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Long talks

Long Talk 1.1

Microenvironment matters – the role of stroma-derived CD44 protein in leukemia drug resistance

Laura Turos-Korgul[1], Aleksandra Ziemińska[1], Jakub Janiec[1], Debjita Mukherjee[2], Magdalena Radecka[2], Jan Guzowski[2], Katarzyna Piwocka[1]

[1] Laboratory of Cytometry, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

[2] Institute of Physical Chemistry, PAS, Warsaw, Poland

Aims: Interactions within the leukemia microenvironment drive the development of drug resistance. We have shown that contact-dependent, tunnelling nanotube-mediated transfer of membrane vesicles from stromal to leukemic cells results in protection of chronic myeloid leukemia (CML) cells from imatinib-induced apoptosis. Proteomic analyses showed that proteins associated with cancer progression, including CD44 (marker crucial for leukemia development), are transported towards leukemia along with vesicles. Presented studies aimed to confirm the tunnelling nanotube-mediated CD44 transfer from stroma to leukemia and elucidate its role in acceptor cells. **Methods**: Fluorescently labelled bone marrow stromal and leukemia cells were co-cultured to study leukemia microenvironment. Using droplet microfluidics, CML and stromal cells were encapsulated in hydrogel microbeads to establish a physiology-mimicking 3D model for studying stroma-leukemia interactions and CD44 transfer. Fluorescent microscopy and flow cytometry were used to assess the level and transfer of CD44. Role of CD44 in drug resistance was examined using rhodamine-123 efflux assay. To check invasive and migratory properties, CD44+ (recipients of stromal CD44) and CD44- leukemic cells were sorted followed by trans-well experiment. **Results**: Co-culture of CML cells with stroma, both in 2D and 3D conditions, increased CD44 levels in leukemia cells, compared to monoculture. The stromal fluorescently-tagged CD44 protein was present within the TNTs, inside cellular vesicles taken by leukemic recipient cells and on the cell membrane of these cells. CML CD44+ recipient cells showed significantly increased efflux pump activity as well as higher invasive and migratory potential compared to CD44-cells (without stromal CD44). Our results show the novel mechanism of microenvironment-mediated protection by direct stroma-derived transfer of CD44 to leukemia. This can play an important role in CML progression and may be a target for potential future therapies.

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Long Talk 1.2

Immune cell composition and functionality in murine glioblastoma microenvironment at single cell and spatial resolution

Karol Jacek [1], Anna M. Lenkiewicz[1], Salwador Cyranowski[1], Mitrajit Ghosh[1], Maria Castro[2], Bozena Kaminska[1]

[1] Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology

[2] Departments of Neurosurgery and Cell and Developmental Biology, University of Michigan Medical School

Malignant gliomas are the most common primary malignant brain tumors in adults. Conventional therapies have not resulted in major improvements in the survival outcomes of malignant glioma patients. Mutations in the *IDH* (coding for Isocitrate Dehydrogenases) play a role in glioma development and progression, particularly in lower-grade gliomas and secondary glioblastomas. The immune microenvironment of gliomas with distinct genetic background has yet to be resolved. In this study, we present the most accurate and comprehensive insights to date into the immune microenvironment of murine experimental gliomas with distinct genetic backgrounds, either wild type or harbouring of the *IDH1* R132H mutation.

To investigate differences in the immune microenvironment composition, phenotype and spatial localization glioma cells with specific genetic defects were implanted, and tumors were dissected at the time of symptoms appearance. CD45+ cells were isolated by flow cytometry and subjected to CITE-seq. Fresh frozen tumor sections were collected in parallel, for Visium Spatial Transcriptomics.

Through the integration of CITE-seq sequencing of CD45+ cells and Spatial Transcriptomics data, we meticulously characterised and described the differences in proportions and phenotypes of 27 immune cell types and states, with a special emphasis on their spatial localization within the tumor microenvironment. Employing Ligand-Receptor analysis, we further elucidated the differences in intricate interplay between myeloid cells and lymphocytes between conditions.

Altogether, our findings hold a promise for the development of targeted immunotherapies in IDH1mt/wt patients, offering multiple potential targets for therapeutic intervention. By unravelling the complexities of the immune-microenvironment in these distinct glioma models, our research contributes valuable insights for future malignant glioma studies and underscores the importance of considering genetic background in immunotherapeutic approaches.

Long Talk 1.3

Interaction between fusion peptide and transmembrane domain of influenza hemagglutinin

Paulina Borkowska[1], Remigiusz Worch[1]

[1] Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Hemagglutinin (HA) is one of the major glycoproteins of influenza virus, which mediates fusion of virus in the late endosomes. The N-terminal part of the HA2 subunit of hemagglutinin acts as fusion peptide (FP) and transmembrane domain (TMD) is located on the C-terminal part. While the fusion activity of FP has been extensively studied, the function of the TMDs during the fusion and its potential interaction with FP remains unclear. In this study we reported the effect of FP, TMDs peptides corresponding to H1 and H3 subtypes and FP:TMDs mixes on the model membrane liposomes. To examine influences of peptides to membranes, we used fluorescence-based experiments including leakage assay, lipid mixing assays and FLIM with lipid order sensitive dye. We showed a lack of synergic interaction between isolated FP and TMD. None of the TMDs exhibited fusion activity or caused disruption in the membrane resulting in leakage. However, notable differences were observed between the H1 and H3 subtypes. Specifically, H1 caused a decrease in the fusion and membrane perturbation activity of FP in lipid mixing and leakage assays, respectively. In contrast, no such interference was observed with H3. Moreover, FLIM results showed that H1 induced an increased order in the membrane, which was the opposite effect compared to FP. To examine the influence of TMD and FP on protein activity, we reconstituted full-length HA in the virus-like particles (VLPs). We observed that the HA activity is boosted when the native TMD H1 sequence was replaced by H3.

Long Talk 2.1

OMA1 protease releases arrested protein import intermediates from mitochondria

Magda Krakowczyk [1], Anna M. Lenkiewicz[1], Tomasz Sitarz[2], Ben Hur Marins Mussulini[3,4], Vanessa Linke[3,4], Dominika Malinska[1], Andrzej Szczepankiewicz[1], Agata Wydrych[1], Hanna Nieznanska[1], Remigiusz A. Serwa[3,4], Agnieszka Chacinska[3,4], Piotr Bragoszewski[1,2]

[1] Laboratory of Protein Homeostasis, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

[2] Centre of New Technologies, University of Warsaw, Warsaw, Poland

[3] IMol Polish Academy of Sciences, Warsaw, Poland

[4] ReMedy International Research Agenda Unit, IMol Polish Academy of Sciences, Warsaw, Poland

Most mitochondrial proteins are synthesized as precursors in the cytosol and require an effective import into the organelle. Such precursor proteins must be largely unfolded to pass through translocation channels in mitochondrial membranes. However, protein misfolding occurs even in physiological conditions in healthy cells. Misfolded proteins can become arrested in translocases, which impair further protein import and mitochondrial function. Both cytosolic and mitochondrial quality control mechanisms can respond to the precursors stuck in a passage. To discover such responses in human cells, we used a model fusion protein designed to stall at an intermediate phase of import. We found out that the degradation of the import-blocking model protein depends on the fitness of mitochondria. In particular, my results revealed that depolarisation of mitochondrial membrane activates the proteolytic processing of model protein by mitochondrial proteases. I identified inner mitochondrial membrane protease OMA1, to be involved in this process. I proved OMA1 cuts model protein inside the mitochondria causing the release of its fragments to the cytosol. The expression of the model protein strongly altered cristae organization by tethering outer and inner mitochondrial membranes. Using transmission electron microscopy I observed that depolarisation induced degradation of the arrested protein restored morphology of mitochondria.

Long Talk 2.2

Motor Neuron Energy Metabolic Reprograming in TDP43 loss-of-function: implication for ALS and FTLD

Ismail Gbadamosi, Ali Jawaid

Laboratory for Translational Research in Neuropsychiatric Disorders, Center of Excellence for Neuronal Plasticity and Brain Disorders, Institute of Experimental Biology Marceli Nencki, Warsaw, Poland.

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD) are devastating neurodegenerative diseases characterized by pathological and molecular overlaps, including the cytoplasmic aggregation of TDP-43 in neurons. Interestingly, certain metabolic conditions, such as type 2 diabetes mellitus and dyslipidemia, have been associated with improved prognosis in ALS and FTLD patients. Our study investigates the molecular interplay between TDP-43 loss of function, a prodromal event in ALS and FTLD, and cellular energy metabolism. Using transcriptomic analysis in NSC-34 mouse motor neurons, we observed significant changes in gene expression related to glucose transport, glycolysis, pyruvate metabolism, and AMPK signalling following TDP-43 loss of function. Moreover, our metabolic assays revealed increased glucose uptake, but disrupted intracellular glucose metabolism, leading to aberrant energy production and enhanced reactive species generation without compensation from the antioxidant defence system. Extracellular metabolic flux analysis indicated a hypermetabolic phenotype in response to TDP-43 loss of function. Notably, our findings also show impaired AMPK metabolic sensing propensity in NSC-34 neurons, suggesting a potential link between TDP-43 dysfunction and dysregulated energy metabolism. In summary, our study uncovers a constellation of metabolic changes resulting from TDP-43 loss of function that may shed light on how dysmetabolic profiles influence the clinical course of ALS and FTLD. Understanding these mechanisms may hold promise for future therapeutic strategies.

Long Talk 2.3

Proteomic approach unravels E3 ligase, TRIM32, as a central mediator β-adrenergic signalling in adipocytes

Katia Al Ghoz, Toufic Kassouf, Grzegorz Sumara

Dioscuri center for metabolic disease, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa, Poland

According to the World Health Organization (WHO), obesity is a pandemic disease that affects >400 million people worldwide. Adipose tissue is the central energy-storing organ that can regulate glucose and lipid metabolism through its signalling effects on the body's major metabolic organs. During energy demands such as exercise, fasting, or cold challenge, stored fat is mobilized by lipolysis to supply the body with free fatty acid and glycerol. β-adrenergic evoked signalling cascades play a major role in the regulation of triglyceride catabolism in adipocytes. Uncontrolled lipolysis can contribute to diabetes and obesity. Efficient protein homeostasis is a critical factor in maintaining normal cell function by regulating various cellular pathways. As the gatekeeper of protein homeostasis, ubiquitin is implicated in almost every cellular pathway described. However, little is known about the importance of ubiquitin in the regulation of metabolism and energy homeostasis during β-adrenergic stimulation in adipose tissue. Therefore, we performed a high throughput proteomic screen using Ubiscan technology to define ubiquitination events that occur upon β-adrenergic stimulation. Among the others, we discovered that the E3 ligase Tripartite Motif family 32 (TRIM32), is activated upon β-adrenergic stimulation of adipocytes. Therefore, by utilizing the gain and loss of function approach we showed that TRIM32 suppresses the activity of AMPK and reduces the lipolysis rate in adipocytes. However, detailed mechanisms mediating TRIM32 activation and action in adipocytes require further investigation. Therefore, we aim to discover the missing elements in our understanding of TRIM32 function in adipose tissue that will allow for more precise targeting of TRIM32 in the treatment of obesity and associated diseases.

Long Talk 3.1

Arc in central amygdala regulates alcohol seeking during cue relapse by changing the synaptic strength

Pegah Taheri, Ahmad Salamian, Narges Sotoudeh, Kasia Radwanska

Laboratory of the molecular basis of behaviour, Nencki institute of experimental biology

Arc is an activity-regulated and highly dynamic protein which is essential in the regulation of the glutamatergic synapses, synaptic plasticity as well as learning and memory. Arc protein promotes internalisation of the AMPA receptors (Chowdhury et al., 2006) and it interacts with NMDA receptors (Nielsen et al., 2019). The role of Arc protein has been proposed in several psychiatric disorders including addiction. In particular, our previous study showed that Arc levels are upregulated in the amygdala during alcohol cue relapse. Moreover, ArcKR mutant mice [with Lysine 268 and 269 mutated to Arginine to prevent Arc ubiquitination and degradation (Wall et al., 2018)] show significantly less alcohol seeking during alcohol withdrawal and cue relapse as compared to wild-type (WT) littermates. However, it is still unclear how Arc contributes to neuronal processes underlying cue-induced alcohol seeking.

In order to track the levels of Arc and Arc ubiquitination during alcohol cue relapse, total Arc and ubiquitinated Arc levels were measured in three time points after cue relapse using WB and ELISA with TUBEs technology. In order to study the role of Arc protein ubiquitination in alcohol-induced synaptic plasticity, Arc protein levels, NMDA receptor subunits and AMPA receptor subunits expression were evaluated in the amygdala of ArcKR and ArcWT mice using Western blot (WB) and synaptic structure was analysed by Golgi staining.

Overall our data support the conclusion that alcohol seeking during cue relapse is regulated by Arc protein ubiquitination and Arc-dependent strengthening of BLA→CeA synapses.

Long Talk 3.2

Training-Induced Neuroplasticity Dynamics in the Motor System of Adult Novice Pianists

Alicja M. Olszewska [1], Maciej Gaca [1], Dawid Droździel [1], Katarzyna Jednoróg [2], Artur Marchewka [1*], Aleksandra M. Herman [1*]

[1] Laboratory of Brain Imaging, Nencki Institute of Experimental Biology, PNAS

[2] Laboratory of Language Neurobiology, Nencki Institute of Experimental Biology, PNAS

* equal contribution senior authors

Piano training is a unique intervention which allows studying neuroplastic processes in the acquisition of complex skills. Here, we uniquely combine longitudinal and cross-sectional designs to explore how the motor system responds in novice pianists over twenty-six weeks of training.

We designed a fine motor task using an MRI-compatible keyboard, performed by novice pianists at multiple time points during training and in skilled musicians once. Task demands varied by playing with both hands either such that contralateral fingers moved simultaneously (symmetrically) or independently (asymmetrically). In the symmetric condition, we observed a training-related decrease in the activation of the insula over the first thirteen weeks, but a gradual increase during the course of training in the parietal, supplementary motor and premotor cortices, cerebellum and striatum. In the asymmetric condition, the activation decreased in the parietal cortex within the first six weeks, followed by the supplementary motor, premotor, insular cortex and cerebellum by thirteen weeks of training, and continuing in the cerebellum until the end of training. Cross-sectional analyses showed that pre-training differences between novices and musicians vanished within twenty-six weeks of training, which suggests that the brain activation of the novices performing the task changed to resemble the musicians by the end of training.

The longitudinal and cross-sectional approaches present converging evidence for training-related plasticity within the motor system of novice pianists. These neuroplastic changes are dynamic and depend on the stage of training and task demands. Moreover, they reflect a shift from attention-based task execution to more automatised movement.

Long Talk 3.3

Sex differences in low-level multisensory integration in developmental dyslexia

Agnieszka Glica[1], Katarzyna Wasilewska[1], Bartosz Kossowski[2], Jarosław Żygierewicz[3], Katarzyna Jednoróg[1]

[1] Laboratory of Language Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteur 3 Street, 02-093 Warsaw, Poland

[2] Laboratory of Brain Imaging, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteur 3 Street, 02-093 Warsaw, Poland

[3]Faculty of Physics, University of Warsaw, Pasteur 5 Street, 02-093 Warsaw, Poland

Reading acquisition involves the integration of auditory and visual stimuli. Thus, low-level audiovisual multisensory integration might contribute to disrupted reading in developmental dyslexia. Although dyslexia is more frequently diagnosed in males, previous studies examining multisensory integration did not evaluate potential sex differences nor tested its neural correlates. In the current study on 88 adolescents, we found that only males with dyslexia showed a deficit in multisensory integration of simple non-linguistic stimuli. At the neural level, both females and males with dyslexia presented smaller differences in response to multisensory compared to unisensory conditions in the N1 and N2 components (early components of event-related potentials associated with sensory processing) than the control group. Additionally, in a subsample of 80 adolescents matched for non-verbal IQ, only males with dyslexia exhibited smaller differences in the left hemisphere in response to multisensory compared to unisensory conditions in the N1 component. Our study indicates that deficits of multisensory integration seem to be more severe in males than females with dyslexia. This provides important insights into sex-modulated cognitive processes that might confer vulnerability to reading difficulties.

Long Talk 5.1

ATP-activated receptors in the pathophysiology of Duchenne Muscular Dystrophy (DMD) – mouse model of DMD

Justyna Róg [1], Dariusz C. Górecki [2], Krzysztof Zabłocki [1]

[1] Laboratory of Cellular Metabolism, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

[2] School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK

Duchenne muscular dystrophy (DMD) is the most common neuromuscular genetic disease. It is caused by mutations in the dystrophin-encoding gene located on chromosome X, leading to a complete lack of full-length (427 kDa) protein. In muscles, dystrophin localises to the sarcoplasmic membrane of muscle fibres and interacts with other proteins of the dystrophin-associated protein complex). This structure increases the mechanical strength of the sarcoplasmic membrane during muscle contraction and is a scaffold for many other proteins. DMD leads to progressive weakness of the skeletal muscles, severe disability and premature death. DMD also affects myoblast, despite the fact that they do not synthesise dystrophin regardless of the mutation in the dystrophin gene because of too early stage of their differentiation. Our data indicate multiple phenotypic changes in myoblast derived from mdx mice (an animal model of DMD). They include deregulated intracellular Ca^{2+} homeostasis i.e. an excessive calcium accumulation and aberrant calcium response to different stimuli. We also observed severe changes in proteins belonging to so-called calcium toolkit such as calcium channels, pumps, transporters and calcium-buffering proteins. In particular, we found an elevated response of myoblasts to ATP which correlates with enhanced expression and activity of purinergic P2Y2 and P2X7 receptors in myoblasts derived from mdx mice compared to dystrophin-positive control. These effects are accompanied by several physiological calcium-dependent effects including affected myoblast proliferation, differentiation and motility. It is worth noting that aberrant calcium homeostasis is also closely related to pathophysiological consequences of DMD in fully differentiated muscles.

Long Talk 5.2

Role MK5 in the Regulation of skeletal muscle biology

Carlos Acosta [1], Katarzyna Kolczyńska[1], Grzegorz Sumara[1]

[1] Laboratory of Metabolic Diseases, Nencki Institute of Experimental Biology, Warsaw, Poland

According to the World Health Organization (WHO) in 2016, more than 1.9 billion adults were overweight and more than 650 million were obese. Obesity predisposes and aggravates several diseases including diabetes, cancer, and musculoskeletal disorders like sarcopenia and dynapenia. Strategies aiming to increase whole-body energy utilization ameliorate obesity and might be beneficial for the improvement of related diseases. Since skeletal muscles are rich in mitochondria, therapies for sarco-obesity focused on increasing substrate utilization and efficient energy consumption represent a strategy of utmost importance. Research conducted in our laboratory revealed that the complex formed by Extracellular signal-regulated Kinase 3 (ERK3) and MAP Kinase-activated kinase 5 (MK5) mediated pathway regulates energy consumption. Deletion of ERK3 in adipocytes inhibits lipolysis but elevates energy dissipation, promoting a lean phenotype. We hypothesize that the ERK3/MK5 complex could represent a targeted therapy for metabolic and skeletal muscle diseases. Thus we aim to decipher the specific role of this kinase complex in the regulation of bioenergetics, mitochondrial biogenesis, and muscle integrity and performance.

Our first results indicate that deletion of ERK3 in skeletal muscle also resulted in elevated energy utilization, lower body weight, and skeletal muscle-protected integrity. To further comprehend its role in human physiology, we have developed mutant strains of mice with overexpression of MK5 and skeletal-muscle specific deletion of MK5, as well as mutant stable skeletal muscle cell lines for in vitro studies. Mice undergo different approaches of exercise intervention experiments with treadmills and grip-strength-meter to study the role of ERK3 and MK5 on skeletal-muscle metabolism, performance, acute response and adaptations to exercise stress stimuli. Our preliminary results indicate that MK5 plays a role in skeletal muscle modulating strength, metabolic phenotype, and exercise capacity..

Long Talk 5.3

Role of stearoyl-CoA desaturase 1 in the regulation of perivascular adipose tissue function

Adrian Sówka [1], Paweł Dobrzyń [1]

[1] Laboratory of Molecular Medical Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Polnad

Vascular disorders are closely associated with obesity. In recent years, perivascular adipose tissue (PVAT) has been identified as a crucial factor in regulating vascular homeostasis. PVAT secretory profile is determined by the metabolic state of adipocytes and its dysregulation is associated with cardiovascular risk. Stearoyl-CoA desaturase 1 (SCD1) is an enzyme that catalyzes the synthesis of monounsaturated fatty acids from saturated precursors. SCD1 knockout mice are resistant to obesity and have improved glucose tolerance and reduced inflammation. However, SCD1 deficiency exacerbates atherosclerosis. Therefore, the aim of our study was to determine the effect of SCD1 deficiency on PVAT adipocyte metabolism. To induce obesity, we fed wild-type (WT) and SCD1 knockout (SCD1-/-) mice with a high-fat diet (HFD). We found that PVAT in SCD1-/- mice has a reduced ability to store lipids when compared to WT likely due to increased content and activity of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). Thin layer chromatography revealed a decreased triacylglycerol pool associated with an increase in free fatty acids content. Moreover, an increase in OXPHOS and uncoupling protein 1 (UCP1) levels was observed in SCD1-deficient adipocytes after HFD. SCD1 deficiency during HFD also resulted in increased mitochondrial cristae density when compared to WT control. Vascular smooth muscle cells treated with conditioned medium derived from SCD1-deficient PVAT adipocytes showed reduced contractile capacity and increased synthetic phenotype markers. Taken together, our data suggest that SCD1 plays a critical role in maintaining vascular homeostasis by regulating metabolism, mitochondrial dynamics and secretory profile in PVAT

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Long Talk 5.4

The role of lipid metabolism and circulating miRNAs in the intergenerational transmission of the effects of parental adverse childhood experiences

Magdalena Gomółka [1], Weronika Tomaszewska [1], Adria-Jaume Roura Canalda [2], Bożena Kamińska-Kaczmarek [2], Ali Jawaid [1]

1 Laboratory for Translational Research in Exposures and Neuropsychiatric Disorders (TREND), Centre of Excellence for Neural Plasticity and Brain Disorder (BRAINCITY), Nencki Institute of Experimental Biology PAS, Warsaw, Poland
2 Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

Adverse childhood experiences (ACE) are associated with detrimental effects on adult physical and mental health. Emerging evidence suggests that behavioral and metabolic perturbations associated with ACE are transmissible across generations. However, the exact mechanisms underlying the effects of ACE on germline for such intergenerational transmission of symptoms remain elusive. Synergizing parallel investigation in a mouse model of ACE induced via unpredictable maternal separation and unpredictable maternal stress (MSUS) and human ACE cohorts, we hypothesize that lipid-associated microRNAs (miRNAs) communicate the effects of ACE to the germline for intergenerational transmission.

small RNA sequencing followed by RT-qPCR revealed overlapping miRNA changes in the serum collected from children, as well as the sperm from adult men with history of ACE. Importantly, the differentially expressed miRNAs were closely connected to lipids both in terms of their transport and regulatory functions.

Parallel investigations in mice involved intergenerational phenotyping after MSUS, as well as lipid-modifying interventions high-fat diet (HFD) and voluntary exercise (VE). Offspring of both MSUS- and HFD-exposed male mice showed impaired glucose tolerance and behavioral deficits. Furthermore, miRNA carriers were isolated from each group and injected into male control and MSUS mice, which were then bred with naïve females. Notably, cross-injections from MSUS into control mice recapitulated the offspring phenotype associated with MSUS, whereas, cross-injections from VE mice into MSUS mice partially mitigated the metabolic phenotype associated with MSUS. Together, these studies provide proof-of-concept for a role of lipids and circulating miRNAs in communicating the effects of ACE to the germline for intergenerational sequelae.

Long Talk 6.1

Combination of Dasatinib and Quercetin rejuvenate the gut-brain axis in aged Wistar rats

Grégory Petrazzo[1], Adam Krzystyniak[1], Małgorzata Węsierska[2], Agnieszka Gadecka[1], Magdalena Dudkowska[1], Anna Bielak- Żmijewska[1], Grażyna Mosieniak[1], Iza Figiel[3], Andreas Dötsch[4], Anke Neidig[5], Sebastian Soukup[5], Claudia Hoffmann[5], Christoph Weinert[5], Daria Guseva[6], Jakub Włodarczyk[3], Ewa Sikora[1]

[1] Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biology, Polish Academy of Sciences

[2] Laboratory of Neuropsychology, Nencki Institute of Experimental Biology, Polish Academy of Sciences

[3] Laboratory of Biophysics, Nencki Institute of Experimental Biology, Polish Academy of Sciences

[4] Department of Physiology and Biochemistry of Nutrition, Max Rubner-Institute - Federal Research Institute of Food and Nutrition, Karlsruhe, Germany

[5] Department of Safety and Quality of Fruit and Vegetables, Max Rubner-Institute - Federal Research Institute of Food and Nutrition, Karlsruhe, Germany

[6] Department of Child Nutrition, Max Rubner-Institute - Federal Research Institute of Food and Nutrition, Department of Safety and Quality of Fruit and Vegetables, Karlsruhe, Germany

Introduction. Cognitive dysfunction negatively impacts the quality-of-life in elders and could sign the onset of dementia. Age-associated senescence is a biological process that progressively alters an organ function and it is known to play a vital role in cognitive decline. Senolytic drugs have been shown to alleviate symptoms of numerous age-related conditions by reducing the organismal senescent burden. **Hypothesis.** Dasatinib and Quercetin (D+Q) senolytic drugs might prevent cognitive decline observed in aged individuals. **Objectives.** We aimed to measure the cognitive performance of aged rats treated with D+Q in behavioural tasks, evaluate the direct mechanistic effects of the senolytics in the brain (synaptic plasticity and epigenetics), and assess systemic effects of the treatment on inflammation and senescence in the gut-brain axis. **Methods.** Aged rats were treated with D+Q or its vehicle for eight weeks and tested in the active allothetic place avoidance task. Synaptic plasticity was measured in hippocampal slices, epigenetic markers were evaluated in hippocampal lysates and inflammation markers in the serum. Microbiota composition was evaluated in the feces, and epithelial tight-junction markers were measured in the gut and brain. **Results.** After D+Q treatment, we observed a long-lasting reduction in age-associated memory impairments supported by changes in synaptic plasticity and the epigenetic landscape. The senotherapy was associated with a reduction in systemic inflammation. We also observed changes in microbiota composition and improvement in the expression of tight-junction markers in the gut and brain. **Conclusion.** Our study brings new insights on the effects of D+Q senolytics in alleviating age-associated cognitive dysfunctions.

Long Talk 6.2

Role of MMP-9 in plasticity of individual dendritic spines

Diana Legutko [1], Piotr Michaluk[1], Ryohei Yasuda[2], Leszek Kaczmarek[1]

[1] Nencki Institute of Experimental Biology, Laboratory of Neurobiology

[2] Max Planck Florida Institute, Neuronal Signal Transduction Laboratory

Understanding the complex network of molecular interactions which underlie morphological and functional synaptic plasticity is a major research challenge. While matrix metalloproteinase-9 (MMP-9) has been implicated in excitatory synaptic plasticity, its precise mechanistic role remains elusive.

To address this, we employed glutamate uncaging (uLTP) to induce long-term structural plasticity in individual synapses within organotypic hippocampal slices. Slices were incubated with MNI-glutamate, and CA1 apical spines were stimulated with 30 laser pulses (0.5 Hz). We measured spine volume changes post-uLTP in the presence of MMP-9 inhibitors and in MMP-9 knockout (KO) conditions. Additionally, fluorescent lifetime imaging (FLIM) of Tropomyosin receptor kinase B (TrkB) and Insulin-like growth factor I receptor (IGFIR) FRET sensors assessed MMP-9's role in neurotrophic factors signalling during LTP induction.

The uLTP protocol induced significant spine growth, indicating structural long-term potentiation (LTP). MMP-9 inhibitors (Inhibitor I and GM 6001) substantially impaired spine growth, as did the absence of MMP-9 in KO neurons. Overexpression of MMP-9 in KO neuronal slices rescued this impairment. Notably, neurotrophic factors receptors activation was reduced without MMP-9 in both IGF-I and BDNF signaling pathways.

In summary, our findings underscore MMP-9's critical involvement in dendritic spine plasticity during LTP initiation, suggesting its fundamental role in neurotrophic factors processing within excitatory synapses.

Long Talk 6.3

Characterisation of regulome in human astrocytes, based on IPSC-derived astrocyte models

Eryk Duński[1], Debadeep Chaudhury[1], Katarzyna Ciuba[1], Aleksandra Piotrowska[1], Bondita Dehingia[1], Aleksandra Pękowska[1]

[1] Dioscuri Centre for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Warsaw, Poland

Human astrocytes feature increased size and enhanced morphological complexity in comparison to their murine and ape counterparts. The coding sequences of genes expressed in the human brain are largely conserved, and it has been proposed that changes in gene expression shape brain evolution in primates. Transcriptional differences between species are mostly a result of changes in the regulome. Thus, to understand the genetic bases of astrocyte evolution, we modelled the evolution of astrocyte transcriptome and regulome by analysing RNA-seq, ATAC-seq and ChIP-seq data from induced pluripotent stem cell (iPSC)-derived astrocytes (iAstrocytes) from human, chimpanzee (Pan Troglodytes) and rhesus macaque (Maccaca mulatta).

Based on those unique datasets, we identified putative enhancers that are only active in human iAstrocytes. We then performed massively parallel reporter assay (MPRA) to confirm their activity. We assayed 5,987 unique sequences, including 3,026 enhancers that are located in proximity of evolutionarily affected genes, 197 enhancers containing single nucleotide (SNP) polymorphisms linked to cognitive ability and brain-related disorders, and 301 enhancers surrounding genes crucial for astrocyte biology. Our results allow us draw conclusions about features of enhancers that drive human-specific expression signatures of astrocytes. We uncover links between evolutionary changes in regulome and human disease.

Long Talk 6.4

Exploring Dendritic Plasticity: How Synaptic and Ion Channel Adaptations Enable

Single Neurons to Rival Artificial Neural Network Complexity.

Ziemowit Sławiński[1], Igor Podolak[2], Yunliang Zang[3] , Eve Marder[4], Daniel Wójcik[1]

[1] Laboratory of Neuroinformatics, Nencki Institute of Experimental Biology PAS, Warsaw, Poland
[2] Faculty of Management and Social Communication , Jagiellonian University, Kraków, Poland
[3] Tianjin University, Tianjin, China
[4] The Marder Lab, The Volen Center for Complex Systems, Brandeis University, Waltham, USA

In the traditional view, we often see a single neuron as less computationally efficient than a multilayer artificial neural network. But is this truly the case? Our investigation delves deep into the computational efficiency of morphologically complex neurons, especially their ability to distinguish between different synaptic patterns. We posed a question: What is the simplest dendritic structure that can master tasks usually reserved for multilayered artificial networks? This exploration not only challenges long-held beliefs about single neuron capabilities but also bridges the gap between biological and artificial neural computation. Furthermore, building upon the foundational homeostatic models pioneered by Eve Marder, we introduced an enhanced model tailored for morphologically complex neurons. Central to our method is the fine-tuning of diverse ion channel composition throughout the whole dendritic tree, ensuring a good balance of homeostatic activity. Our findings reveal that training to recognize synaptic patterns and the homeostatic tuning of ion channels can be unified under one computational strategy. This perspective encourages a more holistic understanding of dendritic tree adaptation, encompassing both synaptic and ion channel modifications. In essence, our study offers a fresh lens through which to understand neuronal learning, merging the worlds of artificial and biological neural networks.

Speed talks

Speed Talk 1.1

Classification of mental disorders from brain waves

Magdalena Szponar [1], Jan Kamiński[1], Bartłomiej Gmaj[2]

[1] Laboratory of Neurophysiology of Mind, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland;

[2] Departament of Psychiatry, Medical University of Warsaw, Warsaw, Poland

Recent years brought an increasing interest in the areas of Machine Learning application in Psychiatry. Many studies show that it is possible to distinguish subjects with a mental disorder from healthy controls, using decoders trained on EEG data, reaching high accuracies over 90%. However, those studies have some significant drawbacks, like small sample sizes and comparing only two groups. In my PhD project, I am attempting to create an algorithm for multiclass classification of mental disorders, using a big archival resting-state EEG database of a psychiatric hospital.

Here I will present the results of a preliminary experiment, in which I classified 8 different groups of mental disorders with multilayer perceptron in One-vs-Rest scheme. Average one-vs-all accuracy on unseen test data was 63%, which is significantly above chance level. Moreover, the accuracy correlated with the value of the activation function of the classifier, showing that we can assess how confident the classification is.

These results show that machine learning classification of mental disorders based on resting state EEG is a promising method, but yet work needs to be done to make it more reliable, so it could be used in clinical practice. I hope that in the future, the algorithm will be an aid for psychiatrists, as an objective and quick method for clinical diagnosis.

Speed Talk 1.2

MK801-enhanced high-frequency oscillations in the piriform cortex are driven by the olfactory bulb in the freely moving rats

Jacek Wróbel [1], Daniel K. Wójcik [1], Mark J. Hunt [1]

[1] Laboratory of Neuroinformatics, Nencki Institute of Experimental Biology, Warsaw, Poland

NMDAR antagonists, at subanesthetic doses, are used to model psychotic-like states in humans and rodents. NMDAR antagonists enhance high-frequency oscillations (HFO) 130-180 Hz recorded in local field potentials (LFP) in many rat brain regions. The olfactory bulb (OB) is an important generator of this activity and may orchestrate this rhythm in other regions. The OB sends powerful excitatory projections to the piriform cortex (PC), and the PC is also known to reciprocally activate the OB. The aim of this study was to examine whether reversible inhibition of the OB or PC affects HFO power in these regions, after NMDAR antagonist (i.p.). Thirty minutes after 0.15 mg/kg MK801 injection, rats received microinfusion of muscimol (0.5 µg) or saline to the OB or PC. A subgroup of rats also received TTX (10 ng) microinfusion to the PC. We found MK801 produced parallel increases in HFO power in the OB and PC, although power was markedly larger in the OB. Muscimol infusion to the OB immediately reduced MK801-enhanced HFO power locally and in the PC. In contrast, muscimol infusion to the PC had only weak effects on HFO in the PC, however, TTX infusion to the PC immediately reduced the HFO power in the PC, without affecting the OB. Together, these findings suggest that HFO recorded in the PC is largely driven by input from the OB and that reciprocal feedback from the PC to the OB does not modulate HFO generated in the OB.

Speed Talk 1.3

Does a mouse need NR2B receptors to feel good?

Justyna Wiśniewska [1], Tomasz Włodarczyk [2], Renata Zakrzewska [2], Anna Beroun [1]

[1] Laboratory of Neuronal Plasticity, BRAINCITY, Nencki Institute of Experimental Biology PAS

[2] Laboratory of Behavioral Methods, Nencki Institute of Experimental Biology PAS

Silent synapses are immature synaptic connections, which are the markers of neuronal plasticity. They express the NMDA but not the AMPA receptor and can arise from pruning of existing synapses (long-term depression, LTD) or be formed *de novo* (long-term potentiation, LTP). Both addictive and non-addictive rewards induce the appearance of silent synapses in the central nucleus of amygdala, which is a structure crucial for assigning the valence of a stimuli.

It is not known whether silent synapses that are formed in the central nucleus of amygdala during the appetitive learning are a result of LTP or LTD.

The aim of this talk is to present the first results describing the effect of inhibition of LTP during the appetitive learning via local knock-out of NR2B subunit of NMDA receptor in the central nucleus of amygdala.

Speed Talk 1.4

The place does matter – differences in neuronal plasticity in hippocampal subregions

Masternak Julia [1], Bączyńska Ewa [1], Bijata Krystian [1], Figiel-Ożóg Izabela [1], Włodarczyk Jakub [1], Bijata Monika [1]

[1] Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology, Polish Academy of Science Pasteura 3, 02-093 Warsaw, Poland

Depression is one of the most widespread illnesses in the world. Among the many symptoms we can observe e.g. anhedonia, chronic fatigue, lack of motivation, low self-esteem or suicidal thoughts. Unfortunately, therapeutic effects of drugs appear after dozen of days of treatment. In order to develop new therapies, a better understanding of the mechanisms regulating this disorder is needed.

During this study we coupled a variety of methods. We have used behavioral evaluation, morphometric analysis of dendritic spines, gel zymography, CDC42 pull-down analysis as well as primary dissociated culture of pyramidal and granule neurons to investigate the 5-HT7R-mediated profiles of activation proteins involved in regulation of actin cytoskeleton in different hippocampal subregions and determining their role in structural plasticity.

We found that short-term activation of 5-HT7R leads to depressive-like behavior in mice. Morphometric analysis of dendritic spines has shown that stimulation of the 5-HT7 receptor leads to an elongation of dendritic spines in CA1. In dentate gyrus subregion the spines maturation was observed. In contrast to morphometric analysis, gel zymography has shown, that the stimulation of 5HT-7 receptor works in a similar way in every hippocampal subregion (CA1, CA3, DG) - the most significant effect was in CA1 and DG subregions. Interestingly, Cdc42 pull-down showed that acute stimulation of 5-HT7 receptor leads to the increased activity of this protein only in CA1 subregion.

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Speed Talk 1.5

Activity of the restrosplenial cortex during navigation using partial cues of different complexity

Urszula Włodkowska [1], Rafał Czajkowski [1]

[1] Laboratory of Spatial Memory, Nencki Institute of Experimental Biology PAS

During the exploration of the environment, animals have a wealth of information available to them, on the basis of which they need to build a representation of their surroundings in a way that ensures their survival. The retrosplenial cortex has been for a long time implicated as an essential part of the spatial memory circuit, although its precise role still needs to be elucidated. We have performed a series of experiments comparing the activity of RSC in mice exposed to different classes of visual cues of varying complexity. In this talk, I will present our recent findings concerning the involvement of the retrosplenial cortex when animals reconstruct the environment from partial cues belonging to different complexity classes. I will talk about how it fits into the view of RSC as a processing hub of the spatial memory circuit.

Speed Talk 1.6

Induced mTOR hyperactivity in dentate gyrus granule cells leads to the impairment of pattern separation in mice

Farzad Khanipour [1], Adam Gorlewicz [1], Ewelina Knapska[1]

[1] Neurobiology of Emotion Lab, BRAINCITY Center of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute of Experimental Biology

Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy in humans, and in addition to seizures, TLE is often linked to cognitive impairments, with episodic memory being a major deficit in this condition. The hippocampus is a major hub for learning and memory and a focal point for seizure propagation in TLE. The granule cells of hippocampal dentate gyrus (DG) region connect via their mossy fiber axonal terminal giant boutons to the apical dendrites of pyramidal neurons in the CA3 region. This physiological synaptic pattern, when disturbed, may lead to the destabilization of the tuned transmission between DG and CA3 regions, disrupting the normal cognitive functions dependent on them. Dispersion of mossy fiber terminal connections is an underrecognized but commonly observed phenomenon in different models of epilepsy or epilepsy-associated disease, in which, mTOR signaling hyperactivity is a commonly reported characteristic. Hence, to study this phenomenon and its association with cognitive deficits further in detail, we developed a TLE mouse model of mTOR hyperactivity with locally specific *Pten* gene knock-out (Δ*Pten*) in DG neurons, predominantly consisted of the excitatory granule cells. Using both structural and functional imaging in conjunction with behavioral studies on Δ*Pten* mice, we demonstrate that hyperactivation of mTOR in DG granule cells does lead to the dispersion of mossy fiber boutons, accompanied by severe disruption of pattern separation, and thus, episodic memory formation. Yet, far from having conclusive results, the upcoming physiological studies may hopefully shed light on the mechanistic understanding of the observed phenomena.

Speed Talk 3.1

Discovery and Characterization of Terpene Synthases Powered by Machine Learning

Raman Samusevich[1,2], Téo Hebra[1], Ratthachat Chatpatanasiri[2], Roman Bushuiev[1,2], Anton Bushuiev[2], Jonáš Kulhánek[2], Tereza Čalounová[1], Adéla Tajovská[1], Milana Perković[1], Josef Šivic[2] and Tomáš Pluskal[1]

[1] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences;

[2] Czech Institute of Informatics, Robotics and Cybernetics of the Czech Technical University in Prague

Terpene synthases (TPSs) generate the core scaffolds of the largest class of natural products, including several first-line medicines. The amount of available TPS sequences is increasing exponentially, but computational characterization of their function remains an unsolved challenge. We assembled a curated dataset of 1k characterized TPS reactions and developed a method to devise highly accurate machine-learning models for functional annotation from the relatively small dataset. Our models greatly outperform existing methods for TPS detection and substrate or product prediction. By applying the models to large protein sequence databases, we discovered and confirmed the activity of seven TPS enzymes previously undetected by state-of-the-art bioinformatic tools. Furthermore, our work describes a new TPS structural domain and distinct subtypes of previously known domains, the discovery of which significantly improved the accuracy of computational predictions. This work demonstrates the potential of machine learning to speed up the discovery and characterization of novel TPSs.

Speed Talk 3.2

The mechanism of the SorC protein family revealed

Markéta Šoltysová [1], Jana Škerlová [1], Petr Pachl [1], Jiří Brynda [1], Jiří Nováček [2], Karel Škubník [2], Michal Babiak [2], Irena Sieglová [1], Milan Fábry [1], Pavlína Řezáčová [1]

[1] Laboratory of Structural Biology, Institute of Organic Chemistry and Biochemistry of Czech Academy of Sciences, Prague, Czech Republic

[2] Cryo-Electron Microscopy and Tomography Core Facility, Central European Institute of Technology, Brno, Czech Republic

Proteins of the SorC family are transcriptional regulators involved in carbohydrate metabolism and quorum-sensing control [1, 2]. The protomers comprise a DNA-binding domain (DBD) and an effector-binding/oligomerization domain (EBD). Based on the sequence of DBDs, the family is divided into two subfamilies, SorC/DeoR and SorC/CggR. So far, structures of only two full-length SorC members and several dissected EBDs have been determined [3-6]. However, no structure of a SorC protein-DNA complex was available, and thus, neither was knowledge of how the proteins recognize their operator targets.

We used an integrative approach of structural biology combining X-ray crystallography and cryo-EM to structurally characterize SorC/DeoR and SorC/CggR prototypes in the complex with their operators. Studies of the full-length repressor-DNA complexes gave us low-resolution information revealing the general mechanism of binding. To gain insight into the inter-atomic contacts, we co-crystallized dissected DBDs and operator's fragments and obtained high-resolution data. Putting all the information together, we propose the SorC family mechanism of the function, which might be used for further basic and applied research.

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Speed Talk 3.3

Split GFP reporter to track factors affecting membrane translocation of ubiquitinated mitochondrial proteins

Krutika Thakkur, Dominika Malinska, Piotr Bragoszweski

Laboratory of Protein Homeostasis, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Mitochondria are associated with many metabolic processes like respiratory energy conversion, biosynthesis of organic compounds, ion homeostasis, cell signaling, and quality control. The synthesis of the majority of mitochondrial proteins occurs in the cytosol with a targeting signal that directs the protein to the mitochondria. Due to their exposure to the cytosolic environment, mitochondria-targeted proteins often undergo modifications before they enter the organelle. Ubiquitination is such modification, which can affect the structure and function of the protein. Our observations indicated that ubiquitin attachment interferes with precursor protein import, although some ubiquitin bound proteins can complete their import.

We generated the split GFP-based reporter system to understand the factors that modulate the transport. The split GFP is expressed as two discrete fragments, and only following their association can the GFP complete its maturation and produce fluorescence. We tested split GFP fragments in fusion with multiple mitochondrial proteins to test their mitochondrial import *in vivo*. We compared such fusions with and without the addition of ubiquitin to mimic the cytosolic stress. The expression of the constructs was confirmed using a western blot. We used cell fractionation to select optimal split GFP-tagged mitochondrial proteins that were optimally enriched in mitochondria. Fluorescent confocal microscopy confirmed that split GFP fragments could assemble to produce fluorescence at the mitochondrial level as the GFP fluorescent signal co-localized with that of the mitochondrial stain Mitotracker. With the optimized reporter, we aim to uncover factors that affect the translocation of proteins after ubiquitination in an unbiased screening approach.

Speed Talk 3.4

Lack of myosin VI affects skeletal muscle energy metabolism

Dominika Wojton [1], Lilya Lehka [1] , Maria Jolanta Rędowicz[1]

[1] Laboratory of Molecular Basis of Cell Motility, Nencki Institute of Experimental Biology, PAS

While studies on muscle function are being intensively performed, new players involved in the regulation of muscle function appear, which role(s) in this process remain(s) enigmatic. One of such player is unconventional myosin VI (MVI). We have demonstrated high expression of this molecular motor in myogenic cells. Moreover, we also showed the impairment of mitochondria homeostasis in MVI-KO myogenic cells. It is well known that mitochondria regulate many critical processes for skeletal muscle physiology and energy metabolism. To address the role(s) of MVI in skeletal muscles metabolism, we performed studies using mice lacking MVI (Snell's waltzer, SV), considered as natural MVI knockouts (MVI-KO). Analysis were performed on muscles derived from different stages of age: newborn, 3- and 12-month-old animals.

We observed upregulation of active form of AMP-activated protein kinase (AMPK), which is a major regulator of energy homeostasis. Taking into consideration that AMPK increases lipid oxidation and inhibits lipid synthesis, we examined the level of triglycerides in skeletal muscle. The analysis revealed significant decrease in their level in 12-month-old MVI-KO mice compared to the control. This observation was accompanied by alteration in the level of proteins involved in lipid metabolism such as FAS, Perilipin 1, pHSL 565, pHSL 563 as well as ATGL. Additionally, we noticed a significant reduction in epididymal fat-to-body weight ratio in MVI-KO mice with respect to control once.

All these imply that lack of MVI may affect the total energy balance. Studies aimed at elucidation of mechanisms underlying this finding are in progress.

Speed Talk 3.5

Mapping evolution of the genetic landscape in primate astrocytes

Debadeep Chaudhury[1], Katarzyna Ciuba[1], Aleksandra Piotrowska[1], Bondita Dehingia[1], Eryk Duński[1], Rüdiger Behr[2], Misbah Abbas[1], Jakub Włodarczyk[3], Marcin Niedbała[4], Wojciech Kaspera[4], Aleksandra Pękowska[1]

[1] Dioscuri Centre for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland

[2] Degenerative Diseases Platform, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany

[3] Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland

[4] Department of Neurosurgery, Medical University of Silesia, 15 Poniatowskiego Street, 40-055 Katowice, Poland

Astrocytes perform a plethora of housekeeping functions in the central nervous system. Additionally, astrocytes are vital for proper synapse function. Hence, astrocytes are likely an important player in brain evolution. But genes and DNA regulatory elements implicated in species-specific astrocyte functions are unclear. To address it, we took advantage of RNA-Seq, ATAC-Seq, ChIP-Seq and HiC to model the evolution of their regulatory landscape. We obtained astrocytes in vitro from induced pluripotent stem cells of humans, chimpanzees, and macaques and sequenced their transcriptome and regulome. From our RNA-Seq data, we found 866 genes showing significant species-specific expression with 65 of them implicated in neurological disorders (NDs), including Alzheimer's disease. Also, based on our ATAC-Seq data, we identified 9097 putative enhancers gained in the human lineage. Over 80% of the evolutionary upregulated genes were closer than 500 kilobases to these human-specific putative enhancers, showing a gain of putative species-specific enhancer activity. Our study reveals genetic changes that could account for the evolved features of the human brain and provide a better mechanistic understanding of NDs.

Speed Talk 5.1

Neural Mechanisms Behind Chronic Stress Resilience

Zeinab Bedrood [1], Tomasz Wójtowicz[1], Monika Bijata[1], Jakub Wlodarczyk [1]

[1] Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology, Warsaw, Poland

Stress resilience is the capacity to endure stress while maintaining normal functioning. Synaptic plasticity-induced neural rewiring leads to resilience. Stress resilience's molecular basis and influencing factors are key in neuropsychiatry and neuroscience. Previous findings of our team showed a strong correlation between stress resilience and altered synaptic protein signaling and 5-HT7 receptor inactivation. Our current research seeks to uncover the neural mechanisms behind it, focusing on protein changes in specific brain regions to explain shifts between resilient and depressive-like behaviors in stress.

For this aim, we conduct research focused on evaluating structural plasticity within the apical dendrites of CA1 pyramidal neurons and granule neurons in the dentate gyrus in mice. The brain slices are treated with an agonist of 5-HT7R and stained to visualize dendritic spine morphology. To achieve this, I performed biolistic fluorescent membranous DiI staining. Then I acquired images from fixed slices using a fluorescence confocal microscope. Subsequently, I investigated the structural plasticity of dendritic spines and analyzed them in a semi-automatically manner, using Spine-Magick software.

Currently I am studying the functional synaptic plasticity namely, mEPSC of CA1 neurons following activation of 5-HT7R. Additionally in order to visualize the dendritic spines, the neurons are filled with biocytin during the patch clamp. In the next step we will investigate the role of the NMDA receptor in the observed changes via pre-incubation with NMDA antagonists - NitroSynapsin or MK-801.

Speed Talk 5.2

Modulation of the injured nerve microenvironment to support axon regeneration in rat newborns.

Beata Kucharz[1], Małgorzata Zawadzka [1]

[1] Laboratory of Neuromuscular Plasticity, Nencki Institute of Experimental Biology, Warsaw, Poland

Peripheral nerves in adult mammals show considerable regenerative capacity. However, neonatal nerve injuries are usually followed by limited regenerative response and loss of motoneurons. The reasons for nerve regeneration failure in newborns are unknown. Nevertheless, it has been reported that grafting of the sciatic nerve segment derived from a more mature rat pup (P5 or older) into a younger one (P3) enables the axons to regenerate into such a conduit which does not happen when the graft comes from a younger or same-aged animal. We aim to understand the mechanisms that regulate the differentiation and subsequent re-differentiation of Schwann cells that occur in response to nerve injury and may support the restoration of functions lost through injury in the mammalian peripheral nervous system.

To this end, we have profiled rat sciatic nerve transcriptome during the postnatal nerve maturation (from the day of birth, P0, to P21) to characterize the dynamics of the gene expression changes associated with development. We found the Wnt/b-catenin signaling pathway to be potentially involved in sciatic nerve maturation. Next, we grafted a sciatic nerve segment from 3- and 6-day-old donors to 3-day-old recipients. Preliminary results of the study revealed differences between Schwann cell response in the recipient sciatic nerve (P3) and the transplanted nerve fragment from an older animal (P6) very early post-injury. We will investigate whether the specific features of Schwann cells derived from the more mature nerve, that we have identified in the grafted nerve segment, might be causative for the efficient regeneration of the injured P3 nerve.

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Speed Talk 5.3

Clock in our brain. The role of temporal processing across different time scales.

Magdalena Stanczyk, Elzbieta Szelag, Klaudia Krystecka, Aneta Szymaszek

Laboratory of Neurophysiology of Mind, BRAINCITY - Center of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Temporal Information Processing (TIP), which refers to our hypothetical internal clock, underlies many cognitive functions. According to the model proposed by Poppel (2009), TIP operates on a few hierarchically ordered time domains. The lowest domain which, operates on tens of milliseconds, constitute a base for the upper domain – hundreds of milliseconds. However, the relations between these two domains, still remain unclear.

This study aimed to verify whether participants characterised by higher efficiency on this basic – tens of milliseconds - domain, will display also higher efficiency on the upper – hundreds of milliseconds domain.

Sixty four young healthy individuals participated in this study. They underwent two kind of tasks: 1) Temporal Order Judgement task to assess their performance on tens of milliseconds domain, and 2) Maximum Tempo Tapping task to measure their performance on hundreds of millisecond domain. Then, based on the results from Temporal Order Judgment task, we selected two groups of participants: characterised by High Level of Performance (HLP) and Low Level of Performance (LLP).

The results showed that HLP achieved faster rate in Maximum Tempo Tapping task than LLP, and synchronised quicker with their internal clock.

This could indicate the strong contribution of a hypothetical internal clock, responsible for temporal organisation and coordination of behaviours across tens and hundreds of milliseconds domains.

Speed Talk 5.4

Measuring individual preferences for diverse tastes in group-housed mice

Mateusz Rycerz, M. Kalinowska, F. Haque, A. Bryksa, J. Sadowska, E. Knapska, A. Puścian

BRAINCITY - Centre of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute, Polish Academy of Sciences, Warsaw, Poland

Understanding what is rewarding to an individual is indispensable for effective influence over their actions. Although much is known about the power of preferred rewards in conditioning behavior, the functional brain underpinnings of the process are still poorly understood.

In the following research, we develop methods for assessment of individual taste preferences in group-housed mice. To that end, we use naturalistic, automated testing in Eco-HAB, an assay recording murine behavior 24h/day. The longitudinal character of the experiments allows testing the changes in preferences over time. We repeatedly exposed animals to two different tastes of candied milk of equal nutritional value and measured the individual intake during time-constrained sessions each day.

We show that mice display individual preferences for diverse tastes of condensed milk. When pretested under single-housing conditions, mice usually start intake from their preferred taste and then swap to the other, showing favor for variability. Further, we show that after being pre-exposed to one taste of condensed milk and subsequently presented with a selection between a familiar and a new taste, mice tend to opt for the latter. Nonetheless, this inclination diminishes over time with repeated instances of choice.

In conclusion, we show that mice have individual preferences for different flavors and that they are more solidified when animals have no contact with other conspecifics. The developed methodological framework will serve for further studies on the neural background of individual preferences, as based on the assessment of voluntary behavior.

Speed Talk 5.5

Tonic GABAAR inhibition in layer 2/3 somatostatin interneurons of somatosensory cortex

Roksana Kapłon, Karolina Bogaj, Joanna Urban-Ciećko

Laboratory of Electrophysiology, Nencki Insitute of Experimental Biology PAS, Warsaw, Poland

Somatostatin (SST) - expressing interneurons are one of the subpopulation of GABAergic interneurons (GABA, Gamma-aminobutyric acid). GABAergic interneurons play an inhibitory role in the neocortex. Tonic GABAA inhibition is mediated by extrasynaptic GABAA receptors and plays an important role in the regulation of the neuronal excitability of mammalian neocortex. However, little is known about the cell-type specific expression of tonic inhibition in particular types of neocortical interneurons. Previous literature study has indicated the lack of tonic inhibition in SST interneurons in the frontoparietal cortex of mice. The aim of the study was to reveal whether layer 2/3 SST interneurons in the barrel cortex (the part of primary somatosensory cortex) of mice express tonic GABAA inhibition and how GABA controls the intrinsic excitability of these interneurons.

We used whole-cell patch-clamp method in acute brain slices prepared from transgenic mice with fluorescently labelled SST interneurons. A tonic current was analysed in layer 2/3 in the somatosensory (barrel) cortex in SST interneurons and neighbouring pyramidal neurons.

We observed that layer 2/3 SST interneurons possess GABAAR mediated tonic inhibition that is comparable to the tonic current in neighbouring pyramidal neurons. Next, we found that SST interneurons express GABAA tonic inhibition that is delta subunit dependent. Finally, we found that GABAARs do not affect the intrinsic excitability of SST interneurons despite the fact that these interneurons are inhibited by extrasynaptic GABAARs.

Altogether, our study indicate that tonic inhibition of SST interneurons might be brain area-specific.

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Speed Talk 5.6

Coming together - the neural dynamics of transition from out-group reserve to in-group fellowship

Yadav A., Rycerz M., Haque F., Bryksa A., Puścian A.

1 Laboratory of Emotions Neurobiology, BRAINCITY - Centre of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute, Polish Academy of Sciences, Warsaw, Poland

Identifying conspecifics as belonging to an in-group, or the social cohort one belongs to, is a quick and unconscious process. In social species, diverse attitudes toward in-group and out-group individuals are reflected in the well-conserved neuronal background. Understanding those brain mechanisms may be facilitated by the behavioral protocols allowing to elicit naturalistic social behavior. To investigate the emergence of social bonds between two unfamiliar groups, we used mice, a species of a highly social nature. Each of the groups, though of the same C57BL6/J strain, came from a different colony. Animals were tested in Eco-HAB, a computer-controlled system mimicking natural murine habitats. The Eco-HAB territory was divided into two equivalent parts - one for each group - which were subsequently merged. From that moment the animals from both groups could freely interact. We show that immediately after the merger animals prefer following in-group conspecifics rather than the out-group ones. Notably, this tendency changes over the following hours. After overcoming initial hesitancy mice start to follow unfamiliar conspecifics with higher frequency than the familiar individuals. Further, in the initial phase after merger animals tend to spend more time with in-group conspecifics. However, to a varying degree, depending on both, the particular group and the individual. In the following hours, the social structure starts shifting, with some mice sticking to their previous social preferences, while others form close relationships with strangers. In summary, we present the data illustrating the process of consolidation of the two previously unfamiliar groups. The presented discoveries form a foundation for further studies of the brain mechanisms underlying novel social bonds.

Speed Talk 6.1

Exploring of the metabolic reprogramming in Hepatocellular carcinoma cells: insights from HepG2 and HepG2/C3A models.

Solmaz Karimi [1], M. R. Wieckowski[1], M. Lebiedzińska-Arciszewska[1]

[1] Laboratory of Mitochondrial Biology and Metabolism, Nencki Institute of Experimental Biology of Polish Academy of Sciences, 3 Pasteur St., 02-093 Warsaw, Poland

Introduction: Energy metabolism plays a critical role in regulating cancer cells' proliferation. To promote cancer cell proliferation, oncogenesis is accompanied by the metabolic shift from mitochondria-driven oxidative phosphorylation to aerobic glycolysis. This metabolic shift is associated with changes in cellular and mitochondrial metabolism resulting from alterations in the levels/activities of key enzymes involved in cellular energetics. Several of them localize permanently or temporarily in mitochondria-endoplasmic reticulum contact sites (MERCs). Our studies aim to investigate the extent of metabolic reprogramming accompanying the shift from the highly proliferative phenotype of HepG2/C3A cells to confluent HepG2/C3A which lose proliferative potential. We also want to investigate MERCs' involvement in HCC metabolism regulation, and the link between metabolic remodelling and MERCs' protein profiles.

Materials and methods: HepG2 and HepG2/C3A cell lines were used. Alteration in proteins' level were examined by western blot. Bioenergetic parameters were evaluated using fluorescent probes.

Results: We observed differences in the growth patterns of confluent HepG2/C3A and HepG2 cells. The metabolic activity of quiescent HepG2/C3A is lower compared to highly proliferative HepG2. HepG2 cells are characterized by increased levels of hexokinase II and OXPHOS subunits, particularly of complexes II and IV in comparison to the HepG2/C3A. Additionally, proliferative and quiescent conditions are characterized by different patterns of the levels/activities of several enzymes involved in metabolic pathways. Moreover, we observed differences in the levels of MERCs' proteins like VDAC, VAPB, SigmaR1, etc.

Conclusions: Our results indicate that metabolic reprogramming is accompanying the shift from proliferative to quiescent phenotype in the HepG2/C3A HCC cellular model.

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Speed talk 6.2

LORA: Lipid Over-Representation Analysis Based on Structural Information

Michaela Vondráčková[1], Dominik Kopczynski[2], Nils Hoffmann[3], Ondřej Kuda[1]

[1] Institute of Physiology, Czech Academy of Sciences, Videnska 1083, 14220 Prague, Czechia

[2]Institute of Analytical Chemistry, University of Vienna, 1090 Vienna, Austria

[3]Forschungszentrum Jülich, Institute of Bio- and Geosciences (IBG-5), 52428 Jülich, Germany

With the increasing number of lipidomic studies, there is a need for efficient and automated analysis of lipidomic data. One of the challenges faced by most existing approaches to lipidomic data analysis is lipid nomenclature. The systematic nomenclature of lipids contains all available information about the molecule, including its hierarchical representation, which can be used for statistical evaluation. The Lipid Over-Representation Analysis (LORA) web application (https://lora.metabolomics.fgu.cas.cz) analyzes this information using the Java-based Goslin framework, which translates lipid names into a standardized nomenclature. Goslin provides the level of lipid hierarchy, including information on headgroups, acyl chains, and their modifications, up to the 'complete structure' level. LORA allows the user to upload the experimental query and universe datasets, select a grammar for lipid name normalization, and then process the data. The user can then interactively explore the results and perform lipid overrepresentation analysis based on selected criteria. The results are graphically visualized according to the lipidome hierarchy. The lipids present in the most over-represented terms (lipids with the highest number of enriched shared structural features) are defined as Very Important Lipids (VILs). For example, the main result of a demo dataset is the information that the query is significantly enriched with 'glycerophospholipids' containing 'acyl 20:4' at '*sn*-2 position'. These terms define a set of VILs (e.g., PC 18:2/20:4;O and PE 16:0/20:4(5,8,10,14);OH). All results, graphs, and visualizations are summarized in a report. LORA is a tool focused on the smart mining of epilipidomics datasets to facilitate their interpretation at the molecular level.

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Speed Talk 6.3

The influence of microbial metabolites on brain immunity

Edyta Bulanda[1], Magdalena Wolska[1], Pilar Rodriguez-Vizo[1], Anna Świątkowska[1] and Tomasz Wypych[1]

[1] Laboratory of Host-Microbe Interactions, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

The gut microbiota is a collection of microbes which inhabit our intestines. It is becoming apparent that the interactions between these microorganisms and human cells are central to maintaining health and become dysregulated in disease. Despite this, one question remains unanswered: how does the gut microbiota influence immune function in distal body organs, such as the brain?

In recent years, we and others have gained important advances towards unravelling the mechanisms that underline the "gut-brain axis", as we pointed to the presence of gut-derived metabolites in this organ. Following up on this, we identified one metabolite which inhibited inflammatory responses in cells from these areas. In glial cells, it ameliorated the production of mediators typically upregulated in multiple sclerosis patients (IL-6, CCL2 and CCL20). Testing the efficacy of metabolite's isomer pointed to the active site of the molecule and allowed us to construct the library of 20 rationally designed chemical derivatives. Some of these modifications exerted a stronger anti-inflammatory effect than the original compound, and some showed a distinct anti-inflammatory profile (i.e. inhibited different mediators). Collectively, these findings constitute the grounds for exploring the efficacy of identified metabolites in vivo and explaining their mechanisms of action.

Overall, our results may constitute the first step towards the development of these compounds as drugs, e.g. in the form of a mixture of identified metabolites, a therapeutic solution that simultaneously targets a broad spectrum of proinflammatory mediators.

Speed Talk 6.4

Heterogeneity and morphology of microglia after transient depletion and repopulation at different ages

Zuzanna M. Luczak-Sobotkowska[1*], Patrycja Rosa[1,2*], Maria Banqueri Lopez[1], Natalia Ochocka[1], Anna M. Lenkiewicz[1], Aleksander Jankowski[2], Bozena Kaminska[1]

[1]Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology of the Polish Academy of Sciences, Warsaw, Poland;

[2]Faculty of Mathematics, Informatics and Mechanics, University of Warsaw, Warsaw, Poland

Microglia, myeloid cells residing in the central nervous system accomplish multiple diverse functions in homeostatic states and in disease. When activated by various stimuli microglia undergo morphological and functional changes manifested by altered gene expression. Survival of microglia depends on colony stimulating factor 1 receptor (CSF1R) signaling. Applying CSF1R inhibitors (i.e. BLZ-945) deplete 99% of microglia in w few weeks and microglia repopulate within 1 week upon cessation of treatment in adult mice. We investigated the origin and functionality of repopulated microglia in young and old mouse brain using single-cell RNA sequencing (scRNA-seq) and immunohistochemistry.

Treatment with BLZ-945 resulted in almost complete microglia depletion (99%) after 21 days. Numbers and density of microglia were restored at day 7 post-treatment, as demonstrated by TMEM119 immunohistochemical staining and flow cytometry. Confocal and Scholl analysis of microglial cell body and branching revealed that repopulated cells display distinct morphology. Using single-cell and bulk RNAseq of immunosorted CD11b+ cells and computational methods we explored if cells restore heterogeneity found in control brains after repopulation.

We present the detailed, single-cell transcriptomic analysis and morphology evaluation of repopulated microglia, which confirm that they reconstitute the functional clusters and vary in morphology. Repopulated microglia in young brain expressed higher levels of pro-inflammatory genes than controls. In old mice more repopulated microglia persist as proliferating cells and do not reach mature microglia phenotype. The results highlight subtle differences in the repopulation of microglia in aged brains that contribute to deterioration of microglial protective functions with age.

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Speed Talk 6.5

Interplay of serum lipids and microglia in the susceptibility to the long-term behavioral effects of adverse childhood experiences

Weronika Tomaszewska[1], Taufik Hidayat[1], Izabela Lepiarz-Raba[1], Magdalena Gomółka[1], Ismail Gbadamosi[1], Aleksandra Cabaj[2], Bartłomiej Gielniewski[2], Ali Jawaid[1]

[1] Laboratory for Translational Research in Neuropsychiatric Disorders (TREND), Nencki-EMBL BRAINCITY Center of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute of Experimental Biology, Warsaw, Poland

[2] Laboratory of Sequencing, Nencki Institute of Experimental Biology, Warsaw, Poland

Adverse childhood experiences (ACE) constitute a major risk factor for neuropsychiatric disorders during adolescence and adulthood. Susceptibility to the long-term behavioural effects of ACE varies across individuals. However, the mechanisms underlying susceptibility vs. resilience to the pervasive effects of ACE remain largely unknown. Emerging evidence supports a role for metabolic factors in susceptibility to the long-term effects of ACE. Here, we propose microglia as the central mediators of such susceptibility and hypothesize that changes in serum lipids and their associated non-coding RNAs induced by ACE can alter microglial functions. For this, we employ a unique multidisciplinary approach that synergizes investigations of samples collected from ethnically diverse human cohorts with in vitro/ex vivo models of human microglia.

Our preliminary investigations demonstrate ACE-induced changes in serum lipids and associated microRNA in children with a recent history of ACE in the form of paternal loss and maternal separation (PLMS). Notably, PLMS children that develop moderate to severe depressive symptoms (PLMS-susceptible) after ACE exhibit decreased high-density lipoproteins (HDLs) and differentially expressed serum microRNAs in comparison to PLMS children with no (or mild) depressive symptoms (PLMS-resilient). Furthermore, treating HMC3 human microglia-like cells with serum from the PLMS-susceptible vs. PLMS-resilient children leads to differential expression of genes involved in glycolysis, as well as functionally disparate phagocytosis. Our ongoing research focuses on validating these findings and studying the impact of samples collected from human ACE cohorts on microglia derived from human induced pluripotent stem cells, as well as microglia-containing human brain organoids.

Posters

Poster 1.1

Cell type specific impact of DDX5 on gene expression and splicing

Misbah Abbas[1], Bondita Dehingia[1], Małgorzata Milewska[1], Marcin Janowski[1], Aleksandra Piotrowska[1], Aleksandra Pękowska[1]

[1] Dioscuri Centre for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland

DEAD-Box RNA Helicase 5 (*DDX5*) contributes to transcriptional regulation and splicing. *Ddx5* inhibits reprogramming and is deregulated in cancer suggesting its role as a gatekeeper of cell identity. We previously found that *Ddx5* impacts genome topology in neural stem (NS) cells. Here, to define the role of *Ddx5* in development, we obtained *Ddx5*$^{-/-}$ embryonic stem (ES) cells and derived NS cells from them. Using RNA-seq and *DESeq2,* we found that the knockout of *Ddx5* had a minor effect on gene expression in ES cells while exerting a profound impact on the NS cell transcriptome. Genes related to the nervous system development, and to RNA processing were frequently upregulated in the *Ddx5*$^{-/-}$ NS cells. Loci implicated in the cell cycle were often downregulated in the knockout cells. Using *rMATS*, we identified exons featuring an altered pattern of splicing upon the removal of *Ddx5.* There were more retained than skipped exons in the *Ddx5*$^{-/-}$ NS cells. Exons aberrantly included in *Ddx5*$^{-/-}$ NS cells had weaker splice sites, and were flanked by larger introns than exons that were more frequently excluded in the absence of *Ddx5*. Protein domains encoded by the exons that were retained in the absence of Ddx5 mediate cell-cell and protein-protein interactions. Altogether, *Ddx5* exerts a differentiation-stage-specific role on the transcriptome. The absence of *Ddx5* activates genes related to neuronal development and promotes aberrant inclusion of exons coding domains mediating protein-protein interactions. Our work reveals the potential mechanisms by which *Ddx5* contributes to the development of the nervous system.

Poster 1.2

Hypoxia shapes the chromatin accessibility changes in glioma-reprogrammed microglial cells.

Monika Dzwigonska[1], Jakub Mieczkowski[2], Bożena Kaminska[1] and Katarzyna Leszczyńska[1]

[1]Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland.

[2]3P-Medicine Laboratory, Medical University of Gdansk, Gdansk, Poland.

Chromatin structure is often dysregulated in cancers, including glioblastoma (GBM). GBM is the most common malignant brain tumor with diffusive growth into the brain, resistance to treatments and a heterogeneous tumour microenvironment (TME). Glioma-associated macrophages and microglia (GAMs) represent a dominant immune cell population in GBM and promote tumour invasion and immunosuppression. In addition, GBM contain hypoxic regions (low in oxygen), which reprogram gene expression, induce invasion and impede the efficacy of major treatments. We recently have shown that hypoxia strongly influences the chromatin accessibility in glioma cells. We hypothesized that it may also impact the chromatin landscape of GAMs accumulating in glioma TME.

Using the ATAC-seq assay on glioma and microglia cell co-culture we determined the genome-wide changes in chromatin accessibility in response to hypoxic stress. In microglia cells, the chromatin accessibility was more affected due to interactions with glioma cells than due to response to hypoxia. Additionally, the interactions with glioma cells increased the chromatin accessibility at genes driving pathways related to cell adhesion and morphogenesis. Adding the hypoxic stress to the glioma-microglia cell interactions resulted in a significant loss of chromatin accessibility in microglial cells in pathways promoting the immune response.

Our data show that hypoxia contributes to the immunosuppressive nature of HGGs by influencing the chromatin openness and cooperates with glioma cell-derived signals. The resulting chromatin accessibility changes affect gene expression in both cell types, and expose critical targets in hypoxic GAMs/glioma cells that could be used to develop new therapy approaches in GBM.

Poster 1.3

The crucial role of HIF-1α and mitophagy in cardioprotection induced by chronic hypoxia

Barbora Opletalova[1], L. Alan[1], R. Bohuslavova[2], K. Holzerova[1], F. Di Lisa[3], G. Pavlinkova[2], F. Kolar[1], P. Alanova[1]

1 Institute of Physiology CAS, Prague, Czech Republic

2 Institute of Biotechnology CAS, Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec (BIOCEV), Vestec, Czech Republic

3 Department of Biomedical Sciences, University of Padova, Padova, Italy

Adaptation to chronic hypoxia (CH), a phenomenon increasing cardiac ischemic tolerance, stimulates the stabilization of HIF-1α transcription factor. This study aims to examine the role of HIF-1α in cardioprotection with special respect to mitochondria using a unique model of heterozygous Hif1-α knockout (Hif1α+/-) mice. Adult male wild type (wt) and Hif1α+/- mice were adapted to CH or kept in normoxia. Physiological responses to CH were assessed and myocardial infarction was induced in isolated perfused hearts. In order to assess mitochondrial characteristics and morphology, mitochondrial respiration measurement, gene and protein expressions, electron microscopy and immunohistochemistry were analyzed in left ventricular myocardial tissue. To monitor mitophagy, microtubule-associated light chain protein 3 assay in the presence or absence of the lysosomal protease inhibitor leupeptin and levels of various proteins were determined. Our results show decreased infarct size in chronically hypoxic wt mice compared to normoxic counterparts. In contrast, this protective effect of CH was absent in Hif1α+/- mice. Moreover, Hif1α haploinsufficiency resulted in changes in mtDNA content, mitochondrial size, respiration, protein expressions. As a possible cause of these changes, mitophagy was evaluated. We found a significant difference in the levels of proteins associated with mitophagy, such as LC3II/LC3I, p62, PINK1, Drp1. Our data suggest that HIF-1α is crucial for CH-induced myocardial protection against I/R injury likely by altering mitochondrial function and activation of mitophagy.

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Poster 1.4

Autophagy inhibition promotes increased exosomes secretion in vascular smooth muscle cells , what imitates senescence phenotype.

Alicja Targońska[1], Karolina Staniak[1,2], Grażyna Mosieniak[1]

1 Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biology PAS, Poland;

2 Laboratory of Biomolecular Interactions Studies, Faculty of Chemistry, Warsaw University of Technology, Poland

Exosomes are secreted by various cell types and contain a diverse cargo of proteins, lipids, and nucleic acids. They act as messengers, delivering their cargo to recipient cells, thereby modulating cell-to-cell communication. Senescence is a state of irreversible growth arrest that can be induced by various stressors, such as DNA damage or telomere shortening. Exosomes derived from senescent cells have been found to promote senescence in neighboring cells, thus propagating the senescent phenotype. Furthermore, specific cargo molecules, packaged within exosomes can modulate senescence-associated secretory phenotype (SASP) and influence the pro- or anti-senescent signaling pathways. Thus the aim of our study was to analyze EVs secreted by human vascular smooth muscle cells (VSMCs) at early and late stage of senescence and correlate it with changes in senescence phenotype. To this end we used the model of VSMCs induced to senescence by doxorubicin treatment and analyzed different senescence markers after 1 and 4 weeks upon senescence induction. Our studies revealed that the level of expression of majority of senescence markers decrease in a time-dependent manner although the number of senescent cells (SA-β-gal (+) and BrdU (-) remained constant. Morover development of senescence phenotype correlated with autophagy inhibition. In contrary, we observed increased level of multivesicular bodies (MV), which participate in exosomes formation and secretion, in late senescent VSMCs. In addition, we have shown that late senescent cells secrete more EVs then young or early senescent VSMCs Moreover, we can modulate the number of secreted exosomes by treating VSMCs with inhibitors of autophagy or mTOR such as bafilomycin A1 or rapamycin. This result indicates that disturbances of autophagy facilitate upregulation of EVs secretion by senescent VSMCs.

Poster 1.5

Exocytosis in astrocytes

Aleksandra Mielnicka [1], Leszek Kaczmarek, Piotr Michaluk

[1] BRAINCITY, Laboratory of Neurobiology, Nencki Institute of Experimental Biology

The Interplay between neuronal circuits and astrocytic processes was described over two decades ago by the term “tripartite synapse”, assumes that neurotransmitters activate metabotropic G_q-associated protein receptors leading to increase in cytosolic Ca^{2+} concentrations in astrocytes. This in turn results in the release of gliotransmitters from astrocytes. To investigate the mechanisms of gliotransmitters secretion is an important step in our attempt to understand the regulation of neuronal transmission.

To examine this phenomenon we use TIRFM to image exocytosis events with Synaptobrevin-2 tagged with pH-dependent GFP probe expressed either in dissociated rat hippocampal and cortical mixed cultures, and in the pure astrocytic ones.

Our results indicate that the rate of spontaneous exocytotic gliotransmission is lower in mixed hippocampal and cortical neuron/glia cultures than in the pure astrocytic. Moreover, electrical stimulation of mixed cultures increases the frequency of exocytotic events even after blocking Ca^{2+} release from the endoplasmic reticulum (ER) using 2-APB and Ryanodine. We have also shown that activation of group I metabotropic glutamate receptors with DHPG in the pure astrocytic culture increases the rate of exocytosis indicating importance of Ca^{2+} released from ER. On the other hand we have shown that using Ca^{2+} chelator-BAPTA decreases the frequency of astrocytic exocytosis in both types of cultures proving importance of extracellular source of Ca^{2+} in gliotransmission. Surprisingly, reducing of neuronal activity with TTX doesn’t affect the rate of exocytosis in mixed cultures.

To conclude, our results indicate that there is a correspondence between presence of neurons, Ca^{2+} signal localization and the efficiency of gliotransmitter release in astrocytes.

Poster 1.6

Protein S-palmitoylation in synaptic plasticity

Agata Pytyś[1], Anna Buszka[1], Izabela Figiel[1], Jakub Włodarczyk[1], and Tomasz Wójtowicz[1]

[1] Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology, Polish Academy of Science, Pasteur Str. 3, 02-093 Warsaw, Poland

S-palmitoylation (S-PALM) is a type of lipid posttranslational modification of proteins. Its reversible nature sets it apart from other types of protein lipidation and enables S-PALM to act as a dynamically-acting molecular switch, akin to phosphorylation, governing the fate of numerous synaptic proteins. In fact it has been estimated that about 40% of the synaptic proteins can undergo S-PALM suggesting a major involvement of this posttranslational modification in regulating the state of the synapse and therefore the ability of the neuronal cells to adequately respond to changes of activity in the network. We aim at delineating S-PALM's involvement in synaptic plasticity of the excitatory synapses of the hippocampus.

To investigate synaptic plasticity-related changes to the protein S-PALM profile we induced chemical long-term potentiation (cLTP) in 14-day-old primary rat hippocampal neurons. We employed two distinct methods of detection of S-palmitoylated proteins: acyl-biotin exchange and click chemistry methods. Induction of cLTP in primary neuronal cultures did not result in any unidirectional shift in S-PALM level of all proteins. In contrast, the S-PALM levels of some, but not all, tested individual synaptic proteins were significantly up- or down-regulated.

Our findings suggest that S-PALM may be involved in the regulatory processes that follow changes in the neuronal network activity and as a result reorganisation of existing synapses and modification of their efficacy. We found that the changes to protein S-PALM levels can occur rapidly (in a matter of minutes) and are protein-specific rather than proteome-wide.

Poster 1.7

Deep learning approaches in observer-independent exploration of cytoarchitectural properties of the non-human primate cerebral cortex

Adam Datta [1], Agata Kulesza [1], Sylwia Bednarek [1], Marcello Rosa [2], Piotr Majka [1, 2]

[1] Laboratory of Neuroinformatics, Nencki Institute of Experimental Biology of the Polish Academy of Sciences, Warsaw, Poland

[2] Department of Physiology and Neuroscience Program, Biomedicine Discovery Institute, Monash University, Clayton, Australia

The cerebral cortex is one of the most characteristic features of the mammalian brain. Considering its laminar structure, the cerebral cortex can be divided into cytoarchitectonically well-defined areas and layers. However, regardless of numerous studies, there is still no clear agreement on its structural and functional subdivision because of the observed variability and method of examination. While that is true, delineation of well-studied areas still depends on extensive neuroanatomical knowledge. To this point, the development and implementation of deep learning techniques ensures additional support for the faster processing of information-rich high-resolution microscopic images and brings a chance to mitigate mentioned obstacles: observer bias and the time-consuming nature of the process.

We propose a U-Net deep learning neural network model for semantic segmentation of the cerebral cortex into cortical layers. This solution was tested using two different datasets: profiles generated from a small sample of brain sections stained with NeuN and Nissl techniques of the whole brain of the non-human primate, the common marmoset monkey (*Callithrix jacchus*). Specifically, we analysed examples of koniocortex, dysgranular, and agranular cortical areas with diverse, clear, and agreed-upon laminar composition.

Our study demonstrated that the U-Net deep learning network model is an adequate tool for detecting the cytoarchitectonic properties of the primate cerebral cortex and automated identification of the individual cortical layers. As a result, it allows for a more robust segmentation, similar to that achieved by expert neuroanatomists, thereby significantly reducing the time required for manual annotation.

Poster 1.8

Imaging white matter fibre density to discriminate between MS and NMOSD

Paweł Jakuszyk[1], Aleksandra Podlecka-Piętowska[2], Bartosz Kossowski[1], Monika Nojszewska[2], Beata Zakrzewska-Pniewska[2], Maciej Juryńczyk[1]

[1] Laboratory of Brain Imaging, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

[2] Department of Neurology, Medical University of Warsaw, Poland

Introduction: Microstructural changes in cerebral normal-appearing white matter are well recognized in multiple sclerosis (MS), but their presence and significance in neuromyelitis optica spectrum disorders (NMOSD) remain unclear. This study aimed to investigate and compare the fiber density (FD) and fiber cross-section (FC) in cerebral white matter between MS, NMOSD, and healthy controls (HC) using fixel-based analysis. Additionally, correlations between FD/FC and clinical disability were assessed.

Methods: Twenty patients with relapsing-remitting MS, 20 patients with AQP-4-IgG-positive NMOSD, and 20 HC underwent a prospective 3-Tesla MRI scan. DWI data were pre-processed and analyzed using MRtrix3. FD, FC, and a combined measure (FDC) were calculated and compared between groups using the general linear model. Clinical and demographic data were obtained from patients' clinical files.

Results: FD and FDC were significantly reduced in MS compared to HC ($p < 0.05$) in visual pathways and cortico-cortical association tracts. FDC showed a minor reduction in optic radiation in NMOSD compared to HC ($p < 0.05$). MS patients had significantly lower FD in the middle longitudinal fascicle compared to NMOSD ($p < 0.05$). Significant negative correlations were observed between EDSS and FD in the cerebellum and between EDSS and FC in corticospinal tracts and corpus callosum in MS patients ($p < 0.05$).

Conclusion: Non-conventional imaging of white matter in the brain suggests presence of fibre damage visual pathways and cortico-cortical association tracts in MS and a potential for fixel-based analysis as a tool that can aid in dinding an imaging discriminator between MS and NMOSD.

Poster 1.9

Role of dopaminergic neurons in substantia nigra in working memory - preliminary study.

Maria Wrzosek[1], Władysław Średniawa[1], Tomasz Pasterski[2], Paweł Sokal[3], Jan Kamiński[1]

[1] Laboratory of Neurophysiology of Mind, Nencki Institute of Experimental Biology, Warsaw, Poland

[2] 1st Military Clinical Hospital with Polyclinic in Lublin - Branch in Elk

[3] Jan Biziel University Hospital Collegium Medicum Nicolaus Copernicus University

Studies on animals and humans have shown that dopamine plays an important role in working memory, although the specific mechanism remains unknown.

One of the possible explanations is that dopamine release stabilizes the ongoing persistent activity and therefore protects working memory from the influx of new, potentially distracting information.

The aim of the current study is to verify this theory by using single neuron recordings of dopaminergic neurons in Substantia Nigra (SN) during a task engaging working memory. It is being conducted on patients with Parkinson's disease undergoing deep brain stimulation (DBS) procedure. During the procedure, the patients are asked to remember and then recall a position of one of the two arrows that are shown in sequence on a screen. Depending on a condition, they either have to recall the first arrow's position and ignore the later ('ignore' condition) or to provide the second arrow's position ('update' condition).

Apart from the recording conducted throughout the procedure, during half of the trials patients' SN was electrically stimulated.

Preliminary findings from the first recording show that the patients' reaction time is significantly longer when the stimulation of SN is applied. However, this difference is only observed in an 'update' condition. Moreover, in some patients a significant difference in a single neurons' activity has been observed during the task after the SN stimulation.

These preliminary results show that we can disrupt the neuronal activity in SN by applying electric stimulation and that it influences the ability to effectively update the information stored in WM.

Poster 1.10

Exploring mouse plasma metabolome and lipidome changes upon chow and high-fat diet

Stanislava Rakusanova, Jiri Hricko, Lucie Rudl Kulhava, Michaela Paucova, Michaela Novakova, Ondrej Kuda, Tomas Cajka

Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 14200 Prague, Czech Republic

Liquid chromatography–mass spectrometry (LC-MS) has become the most applied chromatography−MS tool for analyzing both polar and nonpolar metabolites. A single extraction method or instrumental platform cannot capture the true breadth and scope of polar metabolites (metabolome) and complex lipids (lipidome). Thus, the task is to achieve high metabolite coverage using as few platforms as possible while maintaining the requisite precision and accuracy.

We have developed and validated an LC-MS workflow for the simultaneous extraction of complex lipids and polar metabolites for mouse plasma. The sub-groups of compounds are isolated using an 'all-in-one' extraction with a methanol/methyl tert-butyl ether mixture and water. Analysis of complex lipids is conducted using reversed-phase LC (RPLC) in positive and negative electrospray (ESI) mode, while polar metabolites and exposome compounds are separated using hydrophilic interaction chromatography (HILIC) in ESI(+) and RPLC in ESI(–). Simultaneous acquisition of MS1 and MS/MS spectra in data-dependent mode is employed for each platform. Subsequently, the acquired raw data files are processed using MS-DIAL 4 software.

We applied this workflow to plasma samples from mice studied in systemic energy balance (chow diet) and under chronic nutrient stress (high-fat diet). Overall, 400+ simple and complex lipids and 100+ polar metabolites could be annotated. The main differences were observed for ether-linked phosphatidylcholines (PC) and phosphatidylethanolamine (PE) species, which showed an increase in the high-fat diet group.

Poster 1.11

PKD2 determines lipid-evoked remodeling of LD proteome in small intestine

Magdalena Wit [1], Paolo Ronchi [2], Filip Dziaczkowski [1], Grzegorz Sumara [1]

[1] Dioscuri Centre for Metabolic Diseases, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

[2] Electron Microscopy Core Facility, EMBL, Heidelberg, Germany

Absorption of all macronutrients, including dietary lipids, is largely limited to the small intestine. Epithelial cells lining the organ and mediating calorie uptake (enterocytes) are regularly exposed to high fluctuations in nutrients abundance with each meal ingested. Adaptation to rapidly changing nutritional cues requires an equally fast and precise cellular response to maintain homeostasis. Primarily, lipids are secreted from the intestinal epithelium as chylomicrons into the lymph while a pool of taken taken-up lipids is transiently stored in newly formed lipid droplets (LDs) in enterocytes. However, the factors that determine the distribution of lipids between secretion and storage are largely unknown. Previously we showed that protein kinase D2 (PKD2) promotes intestinal fat absorption via increasing chylomicron size. Recently, we found that PKD2 is implicated also in LD turnover. In the absence of the kinase, LDs are enlarged and the trafficking of LD-degrading enzymes to LDs is impaired. Moreover, we identified that lipid challenge triggers proteolysis of lipases both in vivo and in mouse intestinal organoids but it is prevented in enterocytes depleted from PKD2. Similarly, pharmacological inhibition of chylomicron synthesis preventsthe removal of a targeted protein. Our data indicate that PKD2 integrates lipid secretion and storage pathways in the postprandial period and such a co-regulation is required to optimize lipid uptake. In addition, a newly identified event of lipid-induced proteolysis of LD-associated lipases suggests a potential fine- tuning mechanism of free fatty acids released from intracellular pools in response to dietary lipids load with a limiting role of PKD2.

Poster 1.12

Metabolic profiling of fibroblasts derived from patients suffering from MPAN subtype of Neurodegeneration with Brain Iron Accumulation

Agata Wydrych[1*], Barbara Pakuła[1*], Patrycja Jakubek[1*], Justyna Janikiewicz[3], Aneta M. Dobosz[3], Agnieszka Dobrzyń[3], Marta Skowrońska[2], Magdalena Lebiedzińska-Arciszewska[1#], Mariusz R. Wieckowski[1#]

[1] Laboratory of Mitochondrial Biology and Metabolism, Nencki Institute of Experimental Biology, Warsaw, Poland

[2] II[nd] Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland

[3] Laboratory of Cell Signaling and Metabolic Disorders, Nencki Institute of Experimental Biology, Warsaw, Poland

Introduction: Neurodegeneration with Brain Iron Accumulation (NBIA) is a heterogeneous group of rare diseases, characterized by progressive symptoms associated with excessive iron deposition in the brain. Mitochondrial membrane protein – associated neurodegeneration (MPAN) is a subtype of NBIA caused by mutation in the C19orf12 gene, however, the molecular mechanism underlying MPAN is still not fully understood. The goal of our research is to characterize metabolic alterations and correlate with the clinical phenotype of MPAN patients.

Materials and methods: Cellular and mitochondrial parameters in fibroblasts derived from 11 MPAN patients and 4 healthy donors were evaluated with the use of broad range of experimental approaches and several fluorescent probes. Mitochondrial oxygen consumption was measured with the use of Clark electrode.

Results: Our study revealed several alternations in fibroblasts derived from MPAN patients including e.g., mitochondrial structure and function, autophagy and ROS levels. Differences between disease and healthy metabolic profiles were much better visible under conditions favoring mitochondrial metabolism. Interestingly, affected proliferation (without any signs of cell death) of patients' fibroblasts positively correlates with an altered mitochondrial respiration and increased mitochondrial and cytosolic superoxide levels and clinical phenotype.

Conclusions: Fibroblasts derived from MPAN patients are characterized by the altered cellular proteome as well as affected cellular and mitochondrial metabolism. Increased ROS levels indicate the presence of oxidative stress in patients' fibroblasts. The scale and direction of observed alterations positively correlate with the severity of the disease of individual MPAN patients.

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Poster 1.13

Quantitative analysis of permeability of neurotherapeutics through the *in vitro* blood-brain-barrier using mass spectrometry

Marina Morozovová [1], Jitka Viktorová, Eva Kudová [2]

[1] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 2, 166 10 Prague, Czech Republic

[2] Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, Czech Republic

Permeability through the blood-brain barrier (BBB) is the fundamental element in the design and development of novel drug-like compounds. The main aim of our project is to develop an *in vitro* model for the evaluation of novel neuroactive steroids with improved BBB permeability. Neuroactive steroids are novel compounds of steroid origin without hormonal effect that act as drug-like compounds for the treatment of various central nervous system disorders. As a part of this study, a model of the blood-brain barrier was created *in vitro*. This model consists of three types of cells: i) immortalized human brain microvascular endothelial cells; ii) primary cells of human brain vascular pericytes; and iii) immortalized human astrocytes. Cells were cultured on transwell carrier inserts and transepithelial electrical resistance (TEER) was measured. After reaching a differentiated monolayer, tested neurotherapeutics were added to the apical medium, and after 4h incubation, samples of the apical and basolateral medium were collected. Quantitative analysis of permeability was performed using reversed-phase HPLC with mass spectrometry. Analytes were chromatographically separated using a reverse-phase C18 column, maintained at a temperature of 40°C. This separation was accomplished through the application of a gradient elution program with mobile phase A composed of water, methanol, formic acid (in the ratio of 95:5:1), and acetonitrile (mobile phase B). Calibration curves were linear in the concentration range of 0.5 µM to 80 µM for all measured samples. Development of the method and the results of the permeability of tested commercially available antiepileptics and neuroactive steroids will be presented.

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Poster 1.14

Evaluation of NitroSynapsin on synaptic plasticity in a mouse model of depression

Wing Sze Tse[1], Bartłomiej Pochwat[1, 2], Anna Rafało[2], Bernadeta Szewczyk[2], Bartosz Bobula[3], Krzysztof Tokarski[3], Remigiusz Worch[1], Stuart A. Lipton[4, 5], Monika Zaręba-Kozioł[1], Monika Bijata[1], Jakub Wlodarczyk[1]

[1] Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteur Str. 3, 02-093 Warsaw, Poland

[2] Department of Neurobiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Krakow, Poland

[3] Department of Physiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Krakow, Poland

[4] Neurodegeneration New Medicines Center and Department of Molecular Medicine, The Scripps Research Institute, La Jolla, California 92037, United States of America

[5] Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla, California 92093

Despite being recognised as a major source of disability and mortality worldwide, the search for new and effective pharmacological treatment for major depressive disorder (MDD) has remained a challenge. Traditional therapeutics such as SSRI take time to ameliorate symptoms and a significant percentage of patients do not respond to SSRI treatment. Thus, we evaluated antidepressant potential of NitroSynapsin (NS), an experimental drug and a memantine derivative which antagnoises allosteric N-methyl-D-aspartate receptor (NMDAR) uncompetitively, to address the urgent need for identifying pharmaceutical options with a rapid onset of action and high efficacy but lacking a psychotomimetic effect.

In the present study, we employed the chronic restraint stress (CRS) paradigm to establish a depressive mouse model. Administration of NS evoked antidepressant-like activity in chronically stressed mice. NS reversed CRS-induced behavioural disturbances in the sucrose preference test (SPT) and tail suspension test (TST). Chronic stress induced-morphological changes of dendritic spines, in terms of length/head-width ratio, head width in the medial prefrontal cortex (mPFC) and decrease in the density of dendritic spines in cerebrocortical neurons in the medial prefrontal cortex (mPFC) were significantly restored by NS. This subchronic treatment with NS also prevented CRS-induced reduction in long-term potentiation (LTP) in the mPFC.

These results revealed an antidepressant-like potency of NS for the first time, at the levels of animal behaviour, morphology of dendritic spines and LTP in the mPFC. Further investigations on the mechanism and translation of the findings into the human neuronal model will be carried out.

Poster 1.15

Short-lasting anosmia in rats after gadolinium infusion to the nares: Preliminary findings

Wiktoria Podolecka [1], Mark Jeremy Hunt [1]

[1] Laboratory of Neuroinformatics, Nencki Institute of Experimental Biology of the Polish Academy of Sciences, 02-093 Warsaw, Poland

Anosmia is an olfactory dysfunction which worsens the sense of smell. It is present in many diseases, such as Covid-19, Parkinson's disease or Alzheimer's disease. Most rat models of anosmia are either unreliable or associated with high levels of mortality. Here, we describe a protocol for producing short-lasting, reversible anosmia in adult rats. In this study, either gadolinium (50 µl, 60 mg/ml) or saline (50 µl) was infused bilaterally to the nares. Olfactory function was assessed using a hidden cookie test. Nasal respiration in the olfactory bulb (OB), was measured as a marker of sensory neuron input from the nasal epithelium. Respiratory rhythm (1-10 Hz) was recorded after every conducted hidden cookie test. Finally, histological sections of the nasal epithelium were collected to directly assess damage to the nasal epithelium. We found gadolinium infusion was associated with a marked increase in time to find the hidden cookie compared to control rats, an effect that lasted at least 10 days. Further, gadolinium infused rats had a significant reduction in the respiration rhythm which followed a similar time course to disturbances in the hidden cookie test. Histological analyses revealed damage to the epithelium which was not present in control rats. Together these findings demonstrate that gadolinium infusion to the nares of rats can be used as a safe alternative model to produce anosmia. Our findings suggest that gadolinium produces its effects by reducing excitatory drive of olfactory sensory neurons to the OB.

Poster 1.16

MMP-9 mediates behavioral alterations after bacteria-like inflammation in early life

Karolina Protokowicz [1], Leszek Kaczmarek [1]

[1] Laboratory of Neurobiology, BRAINCITY, Nencki Institute of Experimental Biology, Warsaw, Poland

Various external challenges (e.g., inflammation) during key stages of the nervous system development may contribute to such neurodevelopmental disorders (NDDs) as Autism Spectrum Disorders, ADHD, or schizophrenia. The NDDs tend to last throughout a patient's lifetime and may affect different behavioral domains, including emotions, memory, and sociability. Herein, we have investigated the role of matrix metalloproteinase-9 (MMP-9) an extracellular protease-a key player in plasticity, in inflammation-driven alternations. We decided to focus on the significant period of neurodevelopment – exuberant synaptogenesis, which occurs around postnatal day 7 in mice. During this key stage, we challenged the immune system of C57/BL6 mice with either a single injection of lipopolysaccharide (LPS, 0.05 mg/kg, i.p.) to mimic bacterial infection or physiological saline as a control. Two hours after the administration serum levels of TNF-α, IL-6, IFN-γ, CCL-5, IL-10, and TIMP-1 were elevated in both sexes in comparison to saline-treated control, as shown with Luminex® immunoassay. Additionally, in males (and not females) elevated levels of MMP-9 were observed in serum and the cerebral cortex, as shown also with the gel zymography approach. To further study the role of MMP-9 we conducted a behavioral assessment of adult wild-type animals (MMP-9 WT) and their littermates lacking MMP-9 (MMP-9 KO) after LPS injection in P7. WT males after the immune challenge were less interested in a social odor from unknown animals, while females were more interested in the unknown social object. Interestingly, this effect was not observed in MMP-9 KO animals. In aggregate, the presented results suggest, that MMP-9 is involved in behavioral deficits after immune activation during the critical stage of neurodevelopment.

Poster 1.17

DRD1- and DRD2-positive neurons in the central nucleus of the amygdala for natural and pharmacological reward processing

Łukasz Bijoch[1], Justyna Wiśniewska[1], Tomasz Włodarczyk[2], Teresa Zakrzewska[2], Anna Beroun[1]

[1] Laboratory of Neuronal Plasticity, Nencki Institute of Experimental Biology PAS

[2] Laboratory of Behavioral Methods

The central amygdala (CeA) regulate various emotional behaviors, including fear, anxiety, and pleasure. The CeA comprises several cell types, including neurons expressing dopamine receptors: D1DR and D2DR. Given the limited research on the role of dopamine in the CeA, we studied there the involvement of dopamine-sensitive cells in mouse models of natural (sucrose self-administration) and pharmacological (cocaine injections) reward exposure. Both substances are known to trigger the release of dopamine, but cocaine has an additional effect in blocking the reuptake of this catecholamine.

We aimed to determine how these rewards influence the activity of dopamine-sensitive neurons and whether this modulation leads to plastic changes. Thus, we employed activity-related gene immunohistochemistry, electrophysiological techniques, and two-photon in-vivo calcium imaging. We found that dopamine-sensitive neurons in the CeA are differentially regulated by these rewards. Cocaine increases the activity of D2DR neurons while diminishing the activity of D1DR cells. Conversely, sucrose has the opposite effect on these populations, activating D1DR cells while reducing the activity of D2DR.

Furthermore, we investigated how these neurons contribute to the memory retrieval of cocaine experiences during conditioned place preference (CPP). Interestingly, blocking either D1DR or D2DR cells using chemogenetic methods did not disrupt the animals' preference for the conditioned chamber. However, blocking D1DR neurons surprisingly interrupted the animals' preference on the following day, when the activity of these cells was not artificially manipulated.

Our study provides evidence that dopamine-sensitive cells in the CeA play a role in appetitive behaviors and are differentially regulated during cocaine and sucrose exposure.

Poster 1.18

Selective Modulation of Effort-Based Decision Making by 8-OH-DPAT but not PCPA in Rats

Nathalie Cmarková[2,3], Daniela Kunčická[2,3], Simona Ondráčková[2], David Kačer[4], Daniel Rodriguez[4], Karel Valeš[2,4], Jan Svoboda[2], Aleš Stuchlík[2]

[1] Laboratory of Spatial Memory, Center for Basic and Translational Research in the Field of Biology and Biomedical Sciences, Nencki Institute of Experimental Biology, Poland

[2] Institute of Physiology, Czech Academy of Sciences, Prague, Czechia

[3] Second Faculty of Medicine, Charles University, Prague, Czechia

[4] National Institute of Mental Health, Klecany, Czechia

Decision-making, an executive function crucial for optimal action selection, is widely studied for its potential role as the core of dysfunctional action selection in several neuropsychiatric disorders, such as obsessive-compulsive disorder. These psychiatric disorders associated with decision-making deficits have been linked to abnormal serotonergic systems, thus raising interest in the role of serotonin in decision-making. Our study investigated the impact of an acute treatment by 8-OH-DPAT (0.25 mg/kg) and sub-chronic treatment by PCPA (150 mg/kg) on decision-making behavior in adult male Long-Evans rats, explicitly focusing on their selection of rewarded arm during an effort-based decision-making task in a T-maze apparatus. In addition, high-performance liquid chromatography was performed on the hippocampus, prefrontal cortex, and striatum. Treatment by 8-OH-DPAT resulted in significantly fewer high-reward arm choices and extended the delay to enter the goal arm compared to control animals. In contrast, animals treated with PCPA selected the high reward arm similarly often and with a similar delay as control animals. Treatment with 8-OH-DPAT significantly reduced 5-HIAA and HVA levels in the hippocampus. The PCPA treatment was associated with a 5-HIAA decrease in all structures. However, HVA levels were not affected. The results of the present study indicate that alternations in effort-based decision-making in rats are not associated with the reduction of serotonin levels. This study further showed a different effect of 8-OH-DPAT and sub-chronic PCPA administration on decision-making behavior in rats.

Poster 1.19

Choosing what to choose: a novel method to assess spatial choices.

Julia Świderska[1], Lali Kruashvili[1], Błażej Ruszczycki[2], Katarzyna Radwańska[1]

[1] Laboratory of Molecular Basis of Behaviour, Nencki Institute of Experimental Biology, Warsaw, Poland

[2] Department of Medical Physics and Biophysics, Faculty of Physics and Applied Informatics, AGH University of Krakow, Krakow, Poland

Most aspects of animal behaviour are based on decisions. One of the most extensively researched decisions is spatial choice. This type of decision is understood as a choice that uses spatial information to suppress inappropriate behaviours (Bannerman et al. 2012). The population activity of place cells in the dCA1 area underlies spatial choice and memory. Place cells are best recorded when an animal can move throughout the space freely. However, in the majority of studies on spatial choices, behaviour is investigated with protocols that require direct involvement of the researcher. Additionally, traditional tools used for assessment of spatial choice do not have required dimensions to monitor changes within place cells' activity using genetically encoded, and relatively slow, calcium indicators.

Here, we present a new system for monitoring mice activity and navigation with our recent findings on behavioural protocol that can be employed within it. Apparatus is built of integrated modules including camera system, cue display system, liquid reward dispensers and door control system. Animals are tested within 3 connected corridors, parted with automatic doors, where they can roam undisturbed and consume sweet milk at the end of reward arms from automatic reward dispensers. The automation of our task enables evaluation of spatial choices without the researcher's direct involvement. Additionally, an open construction of the maze allows for recording of the brain cell activity of a freely moving mouse with the use of a miniature microscopy and optogenetic tools along with recording of the animal's behaviour within the entire space.

Poster 1.20

Interaction of CacyBP/SIP and Ribosomal Protein L6 in neuroblastoma NB2a cells

Omid Saberi [1], Małgorzata Maksymowicz [1], Ewelina Jurewicz [1], Anna Filipek [1]

[1] Laboratory of Calcium Binding Protein, Nencki Institute of Experimental Biology, Warsaw, Poland

Introduction: CacyBP/SIP is a protein expressed in various mammalian cells, mainly in neurons and tumour cells. It is a multifunctional protein involved in a wide range of cellular processes. Recent findings, obtained from mass spectrometry, suggest that CacyBP/SIP may interact with some ribosomal proteins such as ribosomal protein L6 (RPL6). Thus, in this work, we verify the interaction between CacyBP/SIP and RPL6.

Methods: 1) Immunoprecipitation assay using NB2a cell lysate and antibody against CacyBP/SIP followed by Western blot using anti-RPL6 antibody. 2) Proximity Ligation Assay (PLA) on NB2a cells with the use of the Duolink *in situ* kit followed by confocal microscopy. 3) *In silico* analysis using ClusPro 2.0 server and YASARA-Structure program.

Results: To confirm CacyBP/SIP-RPL6 interaction, we performed immunoprecipitation and Western blot which revealed that RPL6 is present, along with CacyBP/SIP, in the elution fraction. To detect protein-protein interaction in the cell we performed Proximity Ligation Assay (PLA) and found that CacyBP/SIP and RPL6 are present in close proximity in the cell which endorses the interaction of these two proteins. By applying *in silico* analysis we obtained models of the interacting domains of CacyBP/SIP and RPL6 in ClusPro 2.0 server. The interactions were then evaluated by clustering scores (from ClusPro 2.0), as well as by the contact surface and binding energy (from YASARA-Structure Program). This analysis showed that RPL6 may bind, *via* its stable domain, to the C-terminal fragment of CacyBP/SIP.

Conclusion: Presented results indicate that CacyBP/SIP interacts with RPL6 and thus it may regulate ribosome function.

Poster 1.21

The role of DRC2 protein in cilia beating regulation and interactions between DRC2 and other N-DRC subunits

Salman Ahmadi [1], Sumita Majhi[1], Dorota Wloga [1]

[1] Laboratory of Cytoskeleton and Cilia Biology, Nencki Institute of Experimental Biology, Warsaw, Poland

Motile cilia and eukaryotic flagella are hair-like cell protrusions supported by a microtubule (MT)-based skeleton called the axoneme, composed of 2 singlet MTs surrounded by nine peripheral doublet MTs. Axonemal MTs are accompanied by numerous multiprotein complexes that are arranged in characteristic pattern repeating every 96-nm. The main complexes are outer and inner dynein arms (ODAs and IDAs), radial spokes, and nexin dynein regulatory complex (N-DRC).

The N-DRC connects adjacent doublets and coordinates/regulates the activity of other ciliary complexes including dynein motors. To date, 12 subunits of N-DRC have been identified. To better understand how N-DRC contributes to cilia beating regulation in *Tetrahymena thermophila* I obtained mutant of DRC2 and characterized its motility, and analysed interactions between DRC proteins. The analyses of DRC2-KO mutants showed that lack of DRC2 protein reduces approximately ten times *Tetrahymena* swimming velocity compared to wild type cells. Thus, DRC2, the N-DRC core protein, is crucial for motile cilia activity and cell movement.

Using pull-down assay and overexpressed GFP- or HA-tagged DRC proteins I showed that *Tetrahymena* DRC2 interacts with DRC1, another N-DRC core protein. Moreover, I revealed that DRC1 directly interacts with DRC8 and DRC9, while DRC7 interacts with DRC3 and DRC5. Furthermore, most likely DRC5 also interacts directly with DRC6. Thus, the obtained experimental data are in line with a proposed integrative structural model that has been published recently and shows likely arrangement of DRC proteins within N-DRC complex.

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Poster 1.22

RNA binding proteins contribute to the regulation of architectural function of CTCF during differentiation

Bondita Dehingia [1*], Malgorzata Milewska [1*], Marcin Janowski [1*], Mahmoud Reza Raifee [2], Dietrich Walsh [3], Aleksandra Piotrowska [1], Jacqueline Severino [2], Debadeep Chaudhury [1], Peyman Zare [1], Wolfgang Huber [2], Jianjiang Xu [4], Rafael Casellas [4,5], Timo Zimmermann [3], Jeroen Krijgsveld [2], Aleksandra Pękowska [1,5]

[1] Dioscuri Centre for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland

[2] European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Meyerhofstrasse 1, 69117 Heidelberg, Germany

[3] European Molecular Biology Laboratory Imaging Centre, Meyerhofstrasse 1, 69117 Heidelberg, Germany

[4] Lymphocyte Nuclear Biology, NIAMS, NIH, Bethesda, MD 20892, USA

[5] The NIH Regulome Project, NIH, Bethesda, MD 20892, USA.

*. These authors contributed equally to this work

Mammalian insulatory protein, CTCF, is an eleven-zinc finger DNA-binding protein that shapes the spatial arrangement thereby impacting interactions between enhancers and promoters. The insulatory functions of CTCF are under developmental control but the mechanisms that orchestrate the architectural activity of CTCF are unknown. Here, we took advantage of Selective Isolation of Chromatin Associated Proteins (SICAP-ChIP) to identify CTCF interacting partners in embryonic stem (ES) and neural stem (NS) cells. We reveal a pervasive gain of CTCF – RNA-binding protein (RBP) interactions in the NS compared to the ES cells. We validate this result using proximity ligation assay. We find that CTCF-RBP interactions rely on the presence of RNA. We focus on the DEAD box RNA helicase-Ddx5 (p68) that features an enhanced interaction with CTCF in the NS cells. Using stimulated emission depletion (STED) microscopy and ChIP-seq, we show a differentiation stage-specific effect of Ddx5 on CTCF. Our results reveal that the removal of Ddx5 affects the 3D nuclear distribution of CTCF and CTCF binding to chromatin albeit only in the NS cells. High resolution in-situ Hi-C in NS cells revealed a genome-wide reduction of CTCF-CTCF loop strength in the Ddx5-/- NS cells. Altogether, we discover a pervasive gain of CTCF-RBP interactions upon differentiation of ES cells. Our results reveal fine tuning of CTCF-CTCF loop strengths by RBP, which may be crucial for triggering the maturation of chromatin topology during development.

Poster 1.23

Perceptual Awareness Negativity - does it reflect awareness or attention?

Łucja Doradzińska [1], Michał Bola [1]

[1] Laboratory of Brain Imaging, Nencki Institute of Experimental Biology PAS

A growing body of evidence indicates that Perceptual Awareness Negativity (PAN) - a negative ERP component observed at posterior brain regions around 200 ms after the stimulus presentation - is a robust correlate of phenomenal awareness. However, in our recent opinion paper, we point out that in terms of spatio-temporal features PAN is very similar to the previously described ERP correlates of selective attention (e.g. SN and N2pc; Bola & Doradzińska, 2021). Therefore, whether PAN is indeed a specific mechanism of perceptual consciousness or rather an index of attentional resources engagement remains to be addressed. To this end, we designed a procedure in which stimulus awareness and two aspects of visual attention - exogenous and endogenous - were manipulated orthogonally. Participants were presented with images of faces, which were either backward-masked or unmasked, characterized by a fearful or neutral expression, and defined as targets or task-irrelevant distractors. Our analysis revealed that PAN occurred in response to visible stimuli, in comparison to invisible ones, across all conditions. However, we also found that PAN's amplitude was more negative in response to faces that were fearful (in comparison to neutral) or defined as targets (in comparison to distractors). Therefore, while PAN is indeed robustly related to perceptual awareness, our study suggests that it is modulated by both exogenous and endogenous attention and thus should not be considered a "pure" and specific correlate of consciousness.

Poster 2.1

The influence of microbial metabolites on lung immunity

Magdalena Wolska[1], Pilar Rodriguez-Vizo[1], Edyta Bulanda[1], Anna Świątkowska[1] and Tomasz Wypych[1]

[1] Laboratory of Host-Microbe Interactions, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Gut microbiota is a collection of numerous species, belonging to Eubacteria, Archaebacteria, Protozoa and Fungi kingdoms, that occupy the human gastrointestinal system. While it is recognized that gut microbiota dysbiosis is closely related to loss of homeostasis and human disease, it is not very clear how exactly microbiota affects distal organs, such as the lungs and brain. One of the implicated mediators are microbial metabolites, small chemical compounds derived from gut microbiota.

Preliminary research identified a bacterial metabolite, named here Metabolite X (metabolite's name cannot be revealed due to IP concerns), that was able to inhibit the production of IL-6, CXCL1 and CXCL10 – proinflammatory cytokines and chemokines, characteristic of ARDS (acute respiratory distress syndrome). So far, the immunomodulatory properties have been confirmed in multiple *in vitro* models, including murine-isolated lung cells and alveolar macrophages. These findings warranted evaluating the anti-inflammatory effects in *in vivo* studies and working to identify mechanisms behind the properties of Met X.

ARDS (acute respiratory distress syndrome) develops in patients with severe infections, has a high mortality rate and can cause lifelong impairments. ARDS also features hyperinflammation, which could be a target for future therapies. Overall, our results offer a potential drug with pleiotropic anti-inflammatory attributes and, so far, no detectable side effects.

Poster 2.2

Effects of acute exercise on adipose tissue lipolysis and FAHFA metabolism in relation to obesity and age status

Marko Mitrović[1,2], Olga Horáková[1], Martin Riečan[1], Veronika Kalendová[1,3], Ondřej Kuda[1], Martin Rossmeisl[1]

[1]Laboratory of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic;
[2]First Faculty of Medicine, Charles University, Prague, Czech Republic;
[3]Faculty of Science, Charles University, Prague, Czech Republic.

Exercise is the most effective way of maintaining health, which is partly based on improving adipose tissue function. We have shown that regular exercise in humans raises circulating and adipose tissue levels of fatty acid esters of hydroxy fatty acids (FAHFA), lipokines with potent anti-inflammatory and insulin-sensitizing properties. However, it is unknown how exercise acutely affects adipose tissue lipolysis along with circulating levels of FAHFA and whether this is influenced by metabolic status. We tested this hypothesis in lean, obese and aged C57BL/6J mice subjected to a single bout of treadmill exercise. Exercise-induced lipolysis was impaired in obese and aged mice, as observed at the level of adipose tissue and circulation. Furthermore, adipose tissue explants revealed a greater lipolytic effect in visceral compared to subcutaneous fat. Baseline FAHFA levels in adipose tissue and circulation were dramatically affected by the metabolic status of the animals, which correlated with differences in the expression of genes involved in FAHFA metabolism. Interestingly, acute exercise increased adipose tissue levels of various FAHFA regioisomers only in lean mice, whereas circulating FAHFA levels were mostly unchanged in all groups. In conclusion, lipolysis is attenuated in obese and aged mice, which also affects FAHFA levels in adipose tissue. However, the exact relationship between adipose tissue lipolysis and systemic levels of individual FAHFAs needs further study.

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Poster 2.3

Isolation and characterisation of endothelial cells isolated from dystrophic mice (mdx and mdx^{betageo})

Sabahat Gulzar [1], Krzysztof Milewski, Justyna Róg, Krzysztof Zabłocki[1]

[1]LABORTOARY OF CELLULAR METABOLISM, Nencki institute of Experimental Biology.

Duchenne muscular dystrophy (DMD) is a genetic disorder leading to progressive muscle degeneration due to mutations in the dystrophin-encoding gene. While the muscular consequences of DMD are relatively well characterised, the effects of dystrophin deficiency in endothelial cells are poorly understood. Here, putative effects of mutations in the dystrophin gene were tested with the use of endothelial cells isolated from the brains of mdx and mdx$^{\beta geo}$ mice which are animal models of DMD and from appropriate dystrophin-positive animals. We optimised the method of isolation and cultivation of brain endothelial cells. Their purity was confirmed with the use of the endothelium marker proteins: CD31 and VEGF receptor 2 evaluated by immunocytochemistry (ICC). Next, the western blot analysis was applied to identify dystrophin isoforms in tested cells. We found that control cells as well as endothelial cells from mdx mice express the shortest form of dystrophin (dp71) while no dystrophin was found in cells from mdx^{betageo} mice. Moreover, ICC analysis suggested a reduced level and/or affected distribution of VEGF receptor 2 in mdx cells. Furthermore, we found a substantially lowered level of endothelial nitric oxide synthase (eNOS) in endothelial cells from mdx mice. This observation is in agreement with the previously described reduction of NO generation in muscle endothelial cells from these animals. These results give a solid background for a deeper investigation of the endothelial consequences of DMD.

Poster 2.4

Role of unconventional myosin VI in the heart

Weronika Ficerman [1], Olena Karatsai [1], Maria Jolanta Rędowicz[1]

[1] Laboratory of Molecular Basis of Cell Motility, Nencki Institute of Experimental Biology, Warsaw, Poland

Cardiovascular diseases, including cardiomyopathy (CM), are one of the most common causes of human death. CMs are associated with enlargement and weakening of the heart muscle. One of the factors that could be involved in the development of cardiomyopathy is an actin-based molecular motor, unconventional myosin VI (MVI). Unlike other known myosins, MVI moves towards minus end of actin filaments, functioning as cargo transporter or participating in intracellular compartments organisation.

Our group previously showed that lack of MVI leads to enlargement of the heart already in E14.5 embryos and newborns (P0) of *Snell's waltzer* mice, which are considered as natural MVI knockouts (MVI-KO). As the observed heart enlargement results from increased proliferation but not extensive fibrosis, we decided to study the role of cardiac mesenchymal stem cells (cMSC) in cardiomyopathy development. A fraction of cMSC is c-kit-positive and therefore able to proliferate and differentiate towards cardiomyocytes, vascular smooth muscle cells and endothelial cells. Our studies revealed the highest level of c-kit protein in P0 MVI-KO mice. We have also established a method of c-kit positive cMSC isolation. Increased levels of proliferation markers and c-kit protein in MVI-KO mice suggest that c-kit positive cells may play a pivotal role in development of cardiac hypertrophy.

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Poster 2.5

Unravelling the structural details of the ciliary Nexin-Dynein Regulatory Complex

Sumita Majhi [1], Avrin Ghanaeian [2], Khanh Huy Bui [2], Dorota Wloga [1]

[1] Laboratory of Cytoskeleton and Cilia Biology, Laboratory of Cytoskeleton and Cilia Biology, Nencki Institute of Experimental Biology, Warsaw, Poland

[2] Department of Anatomy and Cell Biology, Faculty of Medicine and Health Sciences, McGill University, Québec, Canada

Motile cilia are microtubule-based cell extensions formed on the apical surface of the epithelial cells lining (in humans) respiratory tract, fallopian tube, and brain ventricles. Ciliary microtubules have characteristic 9x2+2 arrangement - nine outer doublets and a pair of central singlets and serve as docking sites for ciliary complexes. Nexin-dynein regulatory complex (N-DRC) is the main ciliary complex, made up of at least 12 subunits (DRC1-DRC12), linking adjacent outer doublet microtubules and coordinating ciliary beating through intra-ciliary complexes signal transduction. In humans, defects in N-DRC lead to a rare genetic disorder- the primary ciliary dyskinesia, resulting in defective motile cilia causing male and female infertility and respiratory tract infection.

I engineered a number of *Tetrahymena* mutants overexpressing HA- or GFP tagged DRC proteins and using a pull-down assay I verified interactions between DRCs or DRCs and neighbouring proteins. *Tetrahymena* has two DRC4 orthologs, DRC4A and DRC4B. I found that DRC4A and DRC4B preferentially form a heterodimer rather than a homodimer. Moreover, I confirmed that: (i) DRC4 interacts with DRC3 but not with DRC11, (ii) DRC10 comes in contact with CCDC96 protein, a subunit of CCDC96/CCDC113 complex positioned parallel to N-DRC, (iii) CFAP91 lying almost perpendicular to the DRC4A/4B heterodimer makes contact with DRC4B and that (iv) DRC12 is indeed a N-DRC subunit as suggested by an integrated modelling (Ghanaeian *et al*., 2023).

The research was supported by a grant from the National Science Centre, Poland (OPUS21 2021/41/B/NZ3/03612) to DW.

Poster 2.6

Characterization of sofosbuvir polymorphs by polarized Raman microscopy

Věra Schrenková [1,2], Adam Sklenář[1,2], Josef Kapitán[3], Petr Bouř[1], and Jakub Kaminský[1]

[1] Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic

[2] University of Chemistry and Technology, Prague, Czech Republic

[3] Palacký University, Olomouc, Czech Republic

Most drugs are produced in a solid state, but these are often poorly soluble and therefore have a low bioavailability. One way to tackle this problem is to improve its pharmacokinetics by preparing different polymorphs, cocrystals, solvates, or salts of the drug. In pharmaceutic production, it is desirable to monitor such crystalline forms with efficient analytical tools. In this work, we investigate the potential of linearly and circularly polarized Raman microscopy for discrimination of three polymorphic forms of sofosbuvir, an antiviral drug used to treat hepatitis C[1]. To this end, Raman spectra at parallel and perpendicular orientations of linearly polarized light were obtained using a modified Raman microscope equipped with two half-wave plates. Using two quarter-wave plates, we recorded Raman spectra in corotating and contrarotating circular polarization. The resulting spectra include a large signal of low-frequency vibrations close to the laser line, which reflect the intermolecular interactions and packing in the polymorphs[2]. Furthermore, to interpret the observed spectral differences, we employed novel solid-state computational strategies based on density functional theory (DFT). All sofosbuvir forms already gave different unpolarized Raman spectra, but the polarization measurements made the distinction more reliable.

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Poster 2.7

Infrared light modulates activity of mitochondrial large conductance potassium channel

Joanna Jasińska [1], Piotr Bednarczyk[2], Barbara Kalenik[1], Bogusz Kulawiak[1], Antoni Wrzosek[1], Adam Szewczyk[1]

[1] Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw;

[2] Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences -SGGW, Warsaw, Poland

Recently new functions of mitochondria have beed defined. It has been shown that these organelles, despite ATP synthesis and metabolic activity, take part in ROS synthesis, Ca^{2+} buffering and the apoptosis signaling pathway. Mitochondrial potassium channels partially play a role in these functions. Additionally, they are also potential drug targets. Unfortunately, many of potassium channel modulators are not specific. Hence, we propose new mitochondrial channel regulatory mechanism via photobiomodulation. One of the best described light absorbers within the cell is cytochrome c oxidase (COX), an enzyme present in inner mitochondrial membrane. It has four metal centers: the binuclear Cu_A, Cu_B, heme a and heme a3, each of which is capable of absorbing infrared radiation (IR). In our studies we focus on potential functional coupling between COX and mitochondrial large conductance Ca^{2+}-activated potassium channel ($mitoBK_{Ca}$), which would enable regulating channel activity via IR light. In the astrocytoma U87 cell line we carried out patch-clamp experiments with an illumination system. In the first step we oxidized mitochondrial respiratory system by perfusion with ferricyanide. In these conditions $mitoBK_{Ca}$ was inhibited. Illumination with 820 nm wavelength (which is absorption maximum of oxidised Cu_A centre) restored channel activity. This effect was durable. This observation suggests the possible involvement of COX in modulation $mitoBK_{Ca}$ channel activity.

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Poster 2.8

Efficacy of psychotherapeutic interventions for procrastination: Preliminary results of a randomized controlled trial with different cognitive behavioral therapy protocols

Magdalena Pietruch [1], Weronika Browarczyk [2], Joachim Kowalski [3], Jarosław Michałowski [2], Marek Wypych [1]

[1] Laboratory of Brain Imaging, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland

[2] Poznań Laboratory of Affective Neuroscience, SWPS University of Social Sciences and Humanities, Poznań, Poland

[3] Experimental Psychopathology Lab, Institute of Psychology of Polish Academy of Sciences, Warsaw, Poland

Procrastination, a voluntary delay of performing certain tasks despite the negative consequences, affects up to 20% of the general population and leads to anxiety, lowered mood and decreased well-being. Thus, several psychotherapeutic interventions targeting procrastination have been proposed based on findings on its underlying mechanisms. Cognitive behavioral therapy (CBT) appears to be among the most promising ones. However, reliable assessment of the efficacy of different CBT protocols still requires carefully designed studies. In the present four-armed randomized controlled trial with parallel assignment, two CBT protocols were compared with an active control protocol and with a wait-list passive control group. Whereas all three protocols included identical psychoeducation and cognitive intervention modules related to procrastination, they differed in the behavioral intervention modules. In the first one, the behavioral intervention included realistic planning and timely beginning, meanwhile in the second one participants implemented a working time restriction method. The active comparator protocol implemented the Pomodoro time management technique which was believed to not target crucial underlying mechanisms of procrastination. Primary (change in procrastination levels) and secondary (change in depression and anxiety levels) outcomes were assessed before, in the middle and after the treatments. Preliminary, partial results of 138 help-seeking students participating in the ongoing study will be presented. The results of this study will contribute to the evidence for the efficacy and acceptability of the proposed CBT interventions for procrastination as well as will help determine superiority between the behavioral modules of the protocols.

Poster 2.9

Spatial choice in young and aged mice

Monika Puchalska [1], Anna Cały[1], Felix Wäger[2], Lukas Krainer[2], Katarzyna Radwańska[1]

[1] Laboratory of Molecular Basis of Behavior, Nencki Institute of Experimental Biology, Warsaw, Poland

[2] Prospective Instruments LK OG, Dornbirn, Austria

It is widely believed that synaptic plasticity in the hippocampus plays a crucial role in encoding memory and spatial information. Surprisingly, recent data indicate that it is required for spatial choice, i.e. choice that uses spatial information to suppress inappropriate behaviours, rather than spatial memory (Bannerman et al., 2014, 2012). Moreover, data obtained in our laboratory suggests that cellular and behavioural mechanisms that support spatial choice in old mice differ from those observed in young animals (Cały et al., 2021). Since the function of the hippocampus is impaired in aged animals it remains unknown what are the neuronal mechanisms of spatial choice in old mice and how they change in the course of ageing.

In order to study behavioural strategies of aged (>18 months old) and young (3-5 months old) male and female mice used in the process of reaching the reward in a close-to-ecologic conditions, we used the IntelliCage system. To analyse behavioural strategies used by mice to find a reward, we calculated 3 parameters: following (social interactions), patrolling (exploration) and perseveration (cognitive rigidity).

To check the synaptic processes in the dCA1 area that support spatial choice, we stereotactically injected the AAV2.1 coding different variants of PSD-95 protein [wild type and mutant form with Serine 73 substituted by Alanine (PSD95_S73A)] or LV: shRNA to deplete PSD-95 levels. Therefore we want to present how sex, age and synaptic function in dCA1 influence the mices' spatial choice.

Poster 2.10

Cortical Reinstatement: a direct approach to testing hippocampal indexing theory

Katarzyna Sawicka[1], Urszula Włodkowska[1], Monika Falińska[1], Rafał Czajkowski[1]

[1] Laboratory of Spatial Memory, Nencki Institute of Experimental Biology, Polish Academy of Sciences

Episodic memories are crucial elements that determine our sense of identity. Episodic memory formation is mostly dependent on the proper function of the hippocampus. However, mechanisms of episodic memory formation, maintenance and retrieval are only beginning to be elucidated. One of the leading scientific theories about the hippocampal role in memory is indexing theory. This concept builds on the premise that hippocampal neurons are multi-synaptically bi-directionally linked with sensory and associative cortical regions. Nevertheless, it is not clear if cortical reinstating occurs on a neuron-to-neuron basis. We created an artificial pattern of cortical activity by optogenetically stimulating a random population of neurons in the cortical layers of the retrosplenial cortex (RSC) and observed increased c-Fos activity in cells of several structures in the hippocampus. Based on the results, we replayed the hippocampal index by optogenetic activation of Fos-positive neurons in the hippocampus and attempted the in vivo observations of GCaMP signal activity in the retrosplenial cortex. Our ultimate goal is to compare the degree of overlap between the initially activated neurons and the reactivated population in RSC.

Poster 2.11

The Relationship Between Temporal Information Processing and P300 Component

Klaudia Krystecka, Magdalena Stanczyk, Elzbieta Szelag, Mateusz Choinski, Aneta Szymaszek

Laboratory of Neurophysiology of Mind, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Str., 02-093 Warsaw, Poland

Temporal Information Processing (TIP) plays a fundamental role in human cognition as it underlies various cognitive functions, including language, attention, memory, motor control, and planning. Given that TIP is pervasive in shaping human behaviour. Thus, it is reasonable to expect that temporal dynamics are rooted in event-related potential (ERP) components that reflect rhythmic patterns of neural activity. In light of this, we examined relationship between the P300 component and participants efficiency in TIP.

Eighty-one healthy participants (mean age = 25 years) we tested in this study. Participants performed the auditory Temporal-Order Judgement (TOJ) task to assess their efficiency in Temporal Information Processing (TIP). Based on their performance in the TOJ task, they were classified into two groups: more efficient (N = 36) and less efficient (N = 29) timers. Then, they followed a visual Go/No-Go task. P300 was identified on nine electrodes which were then pooled in three lines: frontal(F3, Fz, F4), central(C3, Cz, C4), and parietal(P3, Pz, P4).

The results indicated a significant P300 difference between groups across all pooled regions ($p < 0.05$). More efficient timers displayed greater P300 amplitudes than less efficient timers, which were also significantly associated with better behavioural performance of GO/NO-Go task. Our study contributes to the understanding that temporal resolution is related to the dynamics of EEG activity in normal young adults.

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Poster 2.12

Alterations in Aquaporin 4 (AQP4) immunofluorescence in the hippocampus in a rat model of epilepsy.

Kinga Nazaruk, Kinga Szydlowska, Katarzyna Lukasiuk, Dorota Nowicka

[1] Laboratory of Epileptogenesis, Nencki Institute of Experimental Biology of the Polish Academy of Sciences, 02-093 Warsaw, Poland

The glymphatic system is a mechanism for clearance of brain parenchyma from waste. It consists of perivascular spaces (spaces limited by endothelial cells of blood vessels and perivascular astrocytes) and aquaporin 4-expressing astrocytes. Functionally, it is a brain equivalent of the peripheral lymphatic system. The glymphatic system enables directed fluid movements trough the brain parenchyma. The fluid movement is critically dependent on polarized expression of the water channel aquaporin 4 (AQP4) in astrocytes and is regulated by sleep, anesthesia and circadian rhythm. Disfunctions of the glymphatic system have been shown in several neurological conditions including hydrocephalus, Alzheimer's disease, Parkinson's disease, dementia, multiple sclerosis, traumatic brain injury, stroke, sleep disturbances or aging.

We calculated AQP4 immunoreactivity in astrocytes surrounding blood vessels in the hippocampi at 7 days following proepileptogenic insult, global ischemia evoked by the 4-vessels occlusion. We found that in the hilus the length of AQP4 positive vessels was 114,4±10.1 µm in control and 73,8±5.3 µm ($p<0,0001$) in epileptic animals . AQP4 immunopositive area was: 1691,4± 89 μm^2 in control and 819,2±86,7 μm^2 ($p<0,0001$) in epileptic animals. We did not find significant differences in the density of AQP4 immunoreactivity. Our results suggest the involvement of astrocytic AQP4 polarization and glymphatic system dysfunction in the epileptic brain.

Poster 2.13

The role of the intrinsically disordered regions of MyoD in cell fate determination

Konrad Kowalski [1], Adam Kłosin [1]

[1] Laboratory of Spatial Epigenetics, Nencki Institute of Experimental Biology, Warsaw

During embryonic development, transcription factors (TFs) guide the differentiation of pluripotent blastomeres to specialized cell fates. In addition to a structured DNA-binding domain, most TFs contain intrinsically disordered regions (IDRs), often required for gene activation. Recent studies suggest that the IDRs allow TF to recruit cofactors required for transcriptional initiation by forming phase separated condensates. However, this model has yet to be verified in a developing organism, and the exact mechanisms of how IDRs contribute to the various functions of TFs are still debated. The aim of my doctoral thesis is to determine how the IDRs of a conserved developmental TF MyoD influence its function and its ability to promote phase separation in a model organism *Caenorhabditis elegans*.

The main function of MyoD is activation of genes required for the differentiation of cells into muscle fate during embryogenesis. Research showed that ectopic MyoD expression at an early stage of embryogenesis, causes a complete reprogramming of blastomeres into muscle fate. In a *C. elegans* strain expressing myo-3p::GFP, acting as a muscle marker, the efficiency of MyoD variants can be examined using fluorescence microscopy. Amino-acid sequence analysis suggests presence of two IDRs in MyoD, which flank each side of the centrally placed DNA binding domain. Initial experiments were performed testing variants with each of those IDRs duplicated. Data shows that both duplications alter MyoD's cell reprogramming efficiency by specific to their own way. This suggests that IDRs might play a role in the regulation of MyoD function.

Poster 2.14

Ctcf modulates gene expression in a tissue specific manner.

Małgorzata Milewska-Puchała[1], Aleksandra Piotrowska[1] Debadeep Chaudhury[1], Marcin Janowski[1], Aleksandra Pękowska[1]

[1] Dioscuri Center for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Warsaw, Poland

Gene expression is regulated by cis-regulatory elements (CREs) including promoters, enhancers, silencers, and Ctcf-bound elements termed insulators. Through organizing chromatin into topologically associating domains (TAD), insulators orchestrate interactions between CREs. The Ctcf-bound TAD borders may form architectural loops. TAD borders and architectural loops strengthen upon the differentiation of embryonic stem (ES) to neural stem cells (NS). Remarkably, loops are particularly enhanced around genes crucial for the development of the nervous system.

We took advantage of an auxin-inducible degron (AID) system to acutely deplete Ctcf in ES and NS cells. We observed an overrepresentation of upregulated genes in the NS as compared to ES cells, while the epigenetic signature of chromatin was unaffected. These results indicate that the insulatory functions of Ctcf increase upon loss of pluripotency and neural induction of ES cells.

Aldehyde dehydrogenase family, member A3 (Aldh1a3), regulates retinoic acid signaling pathway which is significant for brain development. We found that the expression of Aldh1a3 increases upon Ctcf depletion but only in the NS cells. We mapped three Ctcf binding sites (CBS) at the Aldh1a3 locus. We knocked them out individually in the ES cells and differentiated into NS cells. Yet, in the NS cells, the deletion only of the 3'-most CBS increase in Aldh1a3 expression. Thus, our data show that Ctcf plays a role in blocking the promoter from activation by the nearby enhancers. We hypothesize that the lack of activation of Aldh1a3 in ES reflects a promoter-enhancer incompatibility at the locus and at this stage of development.

Poster 2.15

The role of lipocalin 2 in the regulation of brain development during prenatal infection

Martyna Pękała [1], Anna Bryksa [2], Ewelina Knapska [2], Leszek Kaczmarek [1], Katarzyna Kalita [1]

[1] Laboratory of Neurobiology, Nencki-EMBL Center of Excellence for Neural Plasticity and Brain Disorders – BRAINCITY, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

[2] Laboratory of Emotions Neurobiology, BRAINCITY - Center of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland

Epidemiological studies indicate that maternal infection during pregnancy is a risk factor for neurodevelopmental disorders; however, the mechanisms underlying this phenomenon remain unclear. One of the proteins highly expressed in the brain in response to infection is lipocalin 2 (Lcn2), an immune protein associated with neurogenesis and synaptic plasticity. Our studies aim to characterize the role of Lcn2 in the regulation of brain development during prenatal infection.

We used a maternal immune activation (MIA) model to mimic maternal infection with i.p. lipopolysaccharide injections in pregnant mice. First, we evaluated Lcn2 mRNA expression in the fetal and adolescent hippocampus. To address how prenatal infection may influence the electrophysiological properties of neurons, we conducted excitability and miniature excitatory postsynaptic currents recordings from hippocampal CA1 neurons. We also performed behavioral experiments to assess anxiety levels and social behavior in MIA offspring.

Our results indicate that Lcn2 mRNA exhibits significant upregulation in the hippocampus following prenatal infection in both the fetal and adolescent brain. Moreover, deletion of the Lcn2 gene led to abnormalities in electrophysiological properties of neurons, accompanied by behavioral impairments, such as elevated anxiety and decreased social behavior. Intriguingly, similar deficits were observed in MIA offspring. However, the absence of the Lcn2 gene in mice exposed to prenatal infection did not lead to further deterioration of the phenotype. These findings suggest that while Lcn2 might play a crucial role in regulating brain development, it does not appear to exert a protective effect during prenatal infection.

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Poster 2.16

Good cells gone bad – the role of SorLA receptor in shaping pro-tumorigenic properties of microglia during glioma progression

Paulina Kaminska[1,2], Mateusz Jakiel[3], Magda Bakun[4], Michal Draminski[3], Michal Dadlez[4], Bozena Kaminska[1], Michal J. Dabrowski[4], Anna R. Malik[2]

Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland
Cellular Neurobiology Research Group, Faculty of Biology, University of Warsaw, Poland
Laboratory of Computational Biology, Institute of Computer Science, Warsaw, Poland
Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, Warsaw, Poland

VPS10P domain receptors are intracellular sorting receptors originally detected in neurons, where they direct protein cargo to their destined subcellular localizations. VPS10P domain receptors can play important roles also in non-neuronal cells including microglia, that acquire diverse functions in healthy and diseased brain. For instance, during glioblastoma progression, microglia along with infiltrating macrophages are reprogrammed into tumor-supportive cells, collectively called glioma associated microglia and macrophages (GAMs). We hypothesize that SorLA (encoded by the *SORL1* gene), a member of the VPS10P family may contribute to tumorigenic properties of GAMs, thereby influencing glioma progression.

To explore if SorLA influences functions of GAMs, we performed bioinformatical analysis of published RNA-seq datasets to characterize *SORL1* expression in subpopulations of human GAMs. Using *in vitro* models we verified if the level of microglial *Sorl1* transcript is regulated by external stimuli and we identified SorLA protein targets. These two approaches indicated that level of *SORL1* expression is linked to pro- or anti-inflammatory activation of GAMs, while proteomic studies revealed protein targets of SorLA in microglia. Finally, *in vivo* experiments demonstrated that SorLA-deficient mice develop smaller gliomas than wild-type animals, which coincides with tumor infiltration by neutrophils, intensified necroptosis and changes in microglia properties. Our results demonstrate that SorLA is a key player in shaping pro-tumorigenic functions of microglia.

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Poster 2.17

Plasma Leptin Surge during Early Postnatal Development as Indicator of Subsequent Body Weight Progression in Mice

Eliska Haasova[1], Kristina Bardova[1], Petra Janovska[1], Olga Horakova[1], Illaria Irodenko[1], Jana Buresova[1], Simona Hajkova[1], Michaela Svobodova[1], Inge Romijnders-van der Stelt[2], Sara Stanic[1], Nivasini Shekhar[1], Jaap Keijer[2], Petr Zouhar[1], Martin Rossmeisl[1], Jan Kopecky[1]

[1]Laboratory of Adipose Tissue Biology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic
[2]Department of Human and Animal Physiology, Wageningen University, Wageningen, The Netherlands

During the perinatal phase, various factors impact energy metabolism and development of adult obesity. Leptin, a key metabolic hormone of adipose tissue, regulates food intake and body weight (BW). Research suggests that postnatal leptin levels may predict obesity susceptibility in mice, suggesting its role in metabolic programming. Our study aimed to assess whether postnatal leptin levels could predict BW variations independently of dietary factors.

We performed two studies using obesity-prone C57BL/6 (B6) and obesity-resistant A/J mice. We measured leptin, adiponectin, lipid markers, gene expression, and leptin secretion adipose tissue explants at 2 and 4 weeks. We then examined their relationship to high-fat diet (HFD)-induced obesity from weeks 12 to 24.

The results showed that B6 mice had higher BW than A/J mice at 2 and 4 weeks. Gender differences emerged at 4 weeks, with females having lower BW. Leptin levels exhibited an inverse pattern, being lower in B6 mice and females at 4 weeks. HFD increased B6 mouse and female A/J mouse BW. Leptinaemia at week 2 significantly correlated with HFD-fed mouse BW. Further analysis using Spearman's correlation coefficients found that only 2-week-old pup's leptin levels correlated with BW. Analysis of leptin secretion from tissue explants revealed that gWAT was the most prominent, but scWAT had the highest absolute secretion. Total leptin secretion matched plasma levels.

In conclusion, postnatal leptin levels can predict dietary-induced obesity predisposition in mice. These findings highlight the link between early metabolic signals and long-term health, advancing our understanding of obesity's developmental origins.

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Poster 2.18

Mitochondrial BK_{Ca} channel in bronchial epithelium damage caused by urban dust

Karolina Pytlak[1], Kamila Maliszewska – Olejniczak[2] , Aleksandra Sęk[1,3] , Adam Szewczyk[1] , Piotr Bednarczyk[2] , Bogusz Kulawiak[1]

[1]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 02-093 Warsaw, Poland

[2]Department of Physics and Biophysics, Institute of Biology, Warsaw University of Life Sciences – SGGW, 02-776 Warsaw, Poland

[3]Faculty of Chemistry, University of Warsaw, 02-093 Warsaw, Poland

Pharmacological activation of mitochondrial potassium (mitoK) channels induces cytoprotective mechanisms. This phenomenon was described during ischemia/reperfusion of brain and heart. Transport of K^+ across the inner mitochondrial membrane is important for regulation of oxidative phosphorylation, reactive oxygen species synthesis and membrane potential. Bronchial epithelium as part of the respiratory system forms an external barrier and is constantly exposed to factors such as pathogens or urban particulate matter (PMs). PMs can damage mitochondria, which leads to epithelial dysfunction. Recently, we have identified a mitoBK_{Ca} channel in the inner mitochondrial membrane of the bronchial epithelial (16HBE14o-, HBE) cells. BK_{Ca} channels are also present in other organelles and in plasmalemma of these cells. We used the CRISPR/Cas9 technology to develop 16HBE14o- cell line with BK_{Ca} α pore-forming subunit knockout (HBE). Loss of the channel changes expression of mitochondrial genes and mitochondrial function. We also observed decrease of monolayer integrity of HBE cells. Additionally, compared the effect of PMs on the survival of HBE wild-type and HBE cells. In conclusion, we want to test whether the mitoBK_{Ca} channel is an effective pharmacological target for cytoprotection against PMs induced damage.

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Poster 2.19

Non-canonical functions of ATP in gene regulation

Sajjad Iqbal[1], Anna Stroynowska-Czerwińska[1], Misbah Abbas[1], Aleksandra Pękowska[1]

[1] Dioscuri Centre for Chromatin Biology and Epigenomics, Nencki Institute of Experimental Biology, Polish Academy of Sciences

ATP is the principal carrier of energy inside the cells. ATP concentration ranges from 8-12 mM, exceeding by a large margin the amount required for energetic processes at any given moment. Recently, ATP has been shown to function as a hydrotrope, a molecule solubilizing organic substances and disrupting protein aggregates. We previously uncovered that depleting ATP by 90%, leads to loss of chromatin loops and the emergences of interactions between cis-regulatory elements. Yet not all the regulatory elements formed contacts upon the depletion of ATP. In this project, we would like to understand what kind of elements pairs are sensitive to the concentration of ATP in the cell. Likewise, we aim to investigate if the hydrotrope-like properties of ATP allow ATP to dissolve interactions between genomic segments. We consider mouse embryonic stem (ES) and neural stem (NS) cells derived from the ES cells. By treating the cells for 4 hours with a glucose analog and oligomycin, which blocks ATP production, we successfully deplete 95% of ATP in both ES and NS cells. Both ES and NS cells withstand this treatment and recover upon release to full medium without these compounds. Next, we implemented Intact-Hi-C, a new technique to map chromatin topology in high resolution established by the Erez Aiden lab at the Baylor College of Medicine. We mapped the alterations in chromatin structure in untreated and ATP-depleted ES cells. We detected chromatin loops built between regulatory sequences. We are currently using this system to address the impact of ATP and its non-hydrolysable analogs on chromatin topology in ES and NS cells.

Poster 2.20

Single-cell transcriptomics reveals the functional heterogeneity of repopulated microglia after pharmacological depletion

Patrycja Rosa [1,2], Zuzanna Łuczak-Sobotkowska[1], Maria Banqueri Lopez[1], Natalia Ochocka[1], Aleksander Jankowski[2], Bozena Kaminska[1]

[1] Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland

[2] Faculty of Mathematics, Informatics and Mechanics, University of Warsaw, Warsaw, Poland

Microglia are myeloid cells residing in the central nervous system, responsible for homeostasis and defense against infections. Previous studies showed their functional heterogeneity manifested by distinct transcriptional profiles of microglia subpopulations and provided insights into their functions. Interestingly, microglia are one of the populations of brain cells which can fully renew during the lifetime of an organism. In this study, we investigated the origin and functionality of repopulated microglia in young and old mice.

Pharmacological treatment with BLZ-945 resulted in complete microglia depletion (<1%) after 21 days, and microglia repopulated within 7 days. Using scRNA-seq of immunosorted CD11b+ cells, we explored if repopulated microglia restore the cell heterogeneity. The analysis of scRNA-seq data showed the presence
of distinct cell clusters with the main myeloid clusters showing similar functionality. The shift in gene expression profiles towards progenitor microglia was suggestive of the premature state of the repopulated microglia. Also, genes engaged in inflammatory processes were expressed at higher levels in the repopulated microglia.

Comparisons between main microglial clusters allowed us to point out the most interesting genes associated in the repopulation process. These genes were involved in signaling pathways and ontologies responsible for cell differentiation and maturation. Comparing microglia repopulation in young (~3 months) and older (~12 months) mice, we observed consistent cell clustering. While there was not major differences in transcriptomic profiles between microglia from control young versus old mice, surprisingly, we noticed that repopulated microglia from old mice were more proliferating and lacked mature microglial cells.

Poster 2.21

Dark side of peripheries - behavioral and fMRI evidence of negative contrast impairment in Retinitis Pigmentosa

Ninghetto M[1], Kozak A[1], Gałecki T[2], Szulborski K[2], Szaflik JP[2], Ołdak M[3], Marchewka A[1], Burnat K[1]

[1]Nencki Institute of Experimental Biology, Warsaw, Poland

[2]Department of Ophthalmology, Medical University of Warsaw, Warsaw, Poland

[3]Department of histology and embryology, Medical University of Warsaw, Warsaw, Poland

In healthy vision, bright slow-motion stimuli are primarily processed by regions of the visual system receiving input from the central part of the scene, while processing of dark fast-motion stimuli is dependent on the peripheral input. We tested 37 Retinitis Pigmentosa patients (RP) with peripheral photoreceptor degeneration and 46 healthy controls. In controls, to model the peripheral loss, we transiently limited visual fields by goggles. To assess the role of the peripheral visual input we measured motion-based acuity thresholds followed by fMRI acquisition with the same stimuli at the individual threshold level. The task involved detecting a circle from an ellipse with matching surfaces, built from random-dot kinematograms in negative or positive contrast. Shapes were centrally located and separated from the background by slow or high motion. During fMRI acquisition, motion-acuity stimuli were presented in blocks and in a 4x4 design: positive or negative contrast and low or high velocity. For all participants, the fast motion in negative contrast was the most difficult. Controls in limited vision had the highest thresholds in fast velocity, irrespectively of the contrast. RP had impaired acuity when measured in negative contrast but not in positive contrast. In RP, in V1, V2 and V3 we found lower responses as compared to controls for the negative contrast, and V1 and dorsal V2-3 areas had a negative BOLD signal. Long-term loss of periphery in RP showed specific impairment of negative contrast stimuli processing, but not in transient loss of periphery in controls, which affected velocity processing.

Poster 2.22

Investigating Water Concentration Dynamics in fMRS Studies

Katarzyna Wasilewska [1], Agnieszka Glica[1], Bartosz Kossowski [2], Katarzyna Jednoróg[1]

[1] Laboratory of Language Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland

[2] Laboratory of Brain Imaging, Nencki Institute of Experimental Biology, Warsaw, Poland

Functional Magnetic Resonance Spectroscopy (fMRS) is a non-invasive technique used to measure changes in metabolite concentrations in response to various stimuli. In order to accurately investigate metabolites, it is essential to consider the influence of the Blood Oxygen Level-Dependent (BOLD) effect.

The purpose of our project is to quantify changes in glutamate concentration within brain regions associated with the reading network during a reading paradigm. The glutamate response function is still unknown, making it important to investigate potential interference from hemodynamic responses.

The fMRS experiment, conducted with unsuppressed water signal acquisition, is similar to the fMRI study. This approach enables us to explore changes in water concentration modulated by the BOLD effect. Notably, water concentration in the brain is approximately 10^4 times higher than that of metabolites. To investigate the BOLD effect, we recruited four volunteers who underwent scanning on a 3 Tesla MRI scanner while performing the same experimental paradigm used in the fMRS study with suppressed water.

Data analysis was performed using the fsl_mrs software, which employs a general linear model. Using knowledge gained from water concentration analysis, we can select appropriate parameters and models to investigate changes in glutamate concentration.