I-01.

ER stress and dysregulation of sphingolipid rheostat in nanoplastic-exposed immature rats

Adamiak K[1,2]., Wencel P[3]., Dabrowska- Bouta B[1]., Sulkowski G[1]., Frontczak-Baniewicz M[4]., Strosznajder R[3]., Struzynska L[1].

[1]Laboratory of Pathoneurochemistry, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Doctoral School of Translational Medicine, Centre of Postgraduate Medical Education, Warsaw, Poland
[3]Laboratory of Preclinical Research and Environmental Agents,,Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[4]Clinical and Research Department of Applied Physiology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: nanoplastic exposure, sphingolipids, ER stress

Due to the global upward trend in the production and consumption of plastic materials, extensive research is underway on environmental sustainability and potential risks to human health. In the environment, plastics degrade into smaller micro- and nanoparticles (MPs/NPs), which pose a great threat to living organisms due to their high reactivity in biological systems. Therefore, the objective of the study was to evaluate the effects of a 3-week exposure of young (two-week-old) Wistar rats to the environmentally-relevant dose of polystyrene nanoparticles (PS-NPs), and to assess their organ accumulation, as well as their role in inducing endoplasmic reticulum (ER) stress and sphingosine-1-phosphate (S1P) dyshomeostasis in the collected tissues (the brain and the liver). The presence of PS-NPs in both tissues was visualized using a confocal microscope. The internalization of particles was much more intensive in the liver than in the brain. TEM analysis revealed ultrastructural changes in both tissues. In the brain, the results revealed features of ER stress and enhanced permeability of the microvessels, while in the liver, depletion of glycogen stores and the presence of lipid droplets were observed. The expression of ER stress markers and enzymes involved in sphingolipid (S1P) homeostasis, specifically sphingosine kinases (Sphk1/2), sphingosine-1-phosphate phosphatase (Sgpp) and sphingosine-1-lyase (Sgpl) was assessed using RT-PCR and/or W-B methods. Overexpression of proteins involved in the induction of ER stress was observed in both tissues, which led to changes in S1P homeostasis. An increase in S1pr3 and S1pr5 expression was observed in the brain, while in the liver, a decrease in Sphk1, Sphk2, and Sgpl mRNA was noted compared to the control, accompanied by an increased expression of Sgpp. Moreover, downregulation of S1P receptors, such as S1pr1 and S1pr3, was revealed. The observed changes indicate tissue-specific modulation of the S1P signaling pathways upon exposure to PS-NPs, which can underlie the enhanced permeability of cerebral microvessels and the disturbed glycogen and lipid deposition in the liver.

The study was financed by the National Science Center, grant no 2021/41/B/NZ7/02183.

I-02.

Selected microRNAs attenuate oxygen–glucose deprivation-induced injury in a reproducible rat primary cortico-hippocampal hypoxia–ischemia model: implications for neonatal hypoxic–ischemic encephalopathy

Bondar M[1]., Mielecki M[2]., Salinska E[1]., Mielecki D[1].

[1]Department of Neurochemistry, Mossakowski Medical Research Institute, Warsaw, Poland
[2]WPD Pharmaceuticals, Warsaw, Poland

Hypoxia–ischemia (HI) remains a major cause of neonatal mortality and can result in hypoxic–ischemic encephalopathy (HIE), damaging the developing brain. HI leads to oxygen and nutrients deprivation, energy failure—primarily ATP depletion—and inflammation, driven largely by glia in affected and penumbral regions, causing neuronal necrosis and apoptosis. Glutamatergic excitotoxicity is a main trigger of HIE development: excessive ionotropic glutamate receptor (iGluR) activation promotes calcium influx, mitochondrial dysfunction, and apoptotic signaling. Neuroprotective strategies based on the NMDA receptor antagonists have failed clinically because of a narrow therapeutic window and severe adverse effects. This underscores the need for safer, mechanism-based therapies targeting downstream injury pathways.

We therefore focused on microRNAs (miRNAs), small noncoding RNAs that regulate gene expression post-transcriptionally and influence proliferation, synaptogenesis, and apoptosis. Specifically, miR-124-3p supports neuronal differentiation, miR-135a-5p synaptic plasticity, miR-335-5p oxidative stress responses, miR-130a-5p antiapoptotic activity, and miR-134-3p dendritic morphology. Our aim was to test selected miRNA mimics and inhibitors in a reproducible in vitro HIE model of primary neuronal culture.

Rat primary cortico-hippocampal cultures were prepared, and eight-day-old neurons were subjected to oxygen–glucose deprivation (OGD). Treatment duration (15 min to 4 h) and media (basal to rich) were varied to establish optimal conditions. Subsequently, the potential beneficial impact of miR-124-3p, miR-135a-5p, and miR-335-5p mimics and miR-130a-5p and miR-134-3p inhibitors was assessed. We measured mitochondrial reduction potential as a cellular survival indicator (MTS), LDH leakage as a necrosis marker, activity of apoptotic factors caspase-3/7, and extracellular glutamate.

A reliable exclusively neuronal rat primary cortico-hippocampal culture model was established with optimal OGD performed in glucose- and antioxidant-free HBSS for 4 h. Preconditioning of OGD-treated cortico-hippocampal neurons with miR-135a-5p, miR-134-3p, and miR-130a-5p significantly attenuated LDH release. Moreover, miR-130a-5p boosted cellular metabolism and suppressed caspase-3/7 activity. All three miRNAs, especially miR-130a-5p, reduced extracellular glutamate. Together, these readouts indicate improved mitochondrial function and less excitotoxic and apoptotic injury, with miR-130a-5p showing the strongest protective profile.

Selected miRNA mimics and inhibitors improved neuronal fitness of rat primary cortico-hippocampal neurons after OGD in an established reproducible model. These findings support investigation of miRNA-based neuroprotection in preclinical models of neonatal HIE.

I-03.

A self-organizing 3D blood-brain barrier spheroid model for studying hypoxic and chemical stress-induced neurovascular alterations

Borkowska A[1]., Romanczuk K[1]., Urbisz A[2]., Chajec L[2]., Pragnaca A[3,4]., Augustyniak K[3]., Malek K[3]., Zdanowski R[1].

[1]Military Institute of Medicine – National Research Institute, Laboratory of Molecular Oncology and Innovative Therapies, Warsaw, Poland
[2]Animal Histology and Embryology Group, Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia, Katowice, Poland
[3]Raman Imaging Group, Faculty of Chemistry, Jagiellonian University, Cracow, Poland
[4]Doctoral School of Exact and Natural Sciences, Jagiellonian University, Cracow, Poland

Keywords: blood-brain barier, 3D spheroid, cellular stress

Blood-brain barrier (BBB) dysfunction is an important component of numerous neurological disorders and may involve alterations in endothelial junctions, transport systems, and interactions between cells of the neurovascular unit. Three-dimensional multicellular models provide an opportunity to investigate these responses within a spatially organized cellular environment.
This study aimed to characterize a human multicellular 3D BBB spheroid model and use it to investigate structural and molecular responses to hypoxic and chemically induced stress.
Spheroids were generated from primary human brain microvascular endothelial cells, astrocytes, and brain vascular pericytes using an agarose-based method. Cellular distribution and spheroid self-organization were evaluated using live-cell fluorescence imaging, including visualization of the internal cellular architecture. Structural and ultrastructural changes were assessed by microscopy and transmission electron microscopy. Barrier-associated permeability was evaluated using 4-kDa dextran, with mannitol used to induce barrier opening. BBB injury was modeled using 1% O2 hypoxia or $HgCl_2$. Expression of tight-junction-associated genes and proteins, efflux transporters, and stress-related markers was assessed by RT-qPCR and Western blot.
The three cell populations spontaneously organized into stable spheroids with a distinct spatial architecture, characterized by preferential localization of endothelial cells at the periphery and astrocytes and pericytes within more internal regions. Low penetration of 4-kDa dextran was observed under control conditions, whereas mannitol significantly increased dextran penetration. Both hypoxia and $HgCl_2$ altered endothelial ultrastructure without causing complete spheroid disintegration. Hypoxia produced the strongest molecular response, significantly decreasing CLDN1, TJP1, and TJP3 expression and reducing occludin and SNAIL protein levels, with a downward trend in ZO-1. Hypoxia also markedly reduced expression of the efflux transporters ABCC1 and ABCG2. In comparison, $HgCl_2$ produced more moderate junction-associated alterations but induced a stronger cytotoxic response accompanied by p62 accumulation.
Human multicellular BBB spheroids reproduce key aspects of neurovascular spatial organization and provide a 3D environment in which distinct responses to pathological stress can be detected. Hypoxia particularly affected junction- and transporter-associated features, whereas $HgCl_2$ induced a different cellular stress profile, supporting the use of this model for investigating mechanisms associated with BBB injury.

I-04.

MOTS-c modulates mitochondrial and metabolic responses of primary rat astrocytes following oxygen-glucose deprivation

Franczak M[1]., Gargas J[1]., Sypecka J[1]., Kawalec M[2].

[1]NeuroRepair Department, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Molecular Biology Unit, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: Oxygen-Glucose Deprivation, MOTS-c peptide, Astrocytes

Oxygen-glucose deprivation is an established in vitro model of hypoxic-ischemic injury that induces profound metabolic disturbances in astrocytes. The aim of this study was to evaluate the effects of the mitochondrial peptide MOTS-c on the bioenergetic response of primary rat astrocytes following oxygen-glucose deprivation.

Primary astrocytes were isolated from mixed glial cultures obtained from neonatal Wistar rat brains. Cells were exposed to 40 minutes of oxygen-glucose deprivation and analyzed immediately after the procedure or following 24 hours of reoxygenation. Cellular bioenergetics were assessed using the Seahorse Mito Stress Test. Oxygen consumption rate and extracellular acidification rate were normalized to cell number. MOTS-c was applied at concentrations of 1, 5, and 10 micromolar. Basal respiration, maximal respiration, acute metabolic response, and non-mitochondrial respiration were evaluated.

Immediately after oxygen-glucose deprivation, basal respiration was reduced in comparison with control cells, particularly in the absence of MOTS-c and following treatment with 5 micromolar MOTS-c, indicating an early suppression of mitochondrial activity after metabolic stress. In contrast, after 24 hours of reoxygenation, astrocytes subjected to oxygen-glucose deprivation without MOTS-c showed increased basal respiration and extracellular acidification compared with corresponding control cells, suggesting a later metabolic adaptation. MOTS-c modified this response in a concentration-dependent manner. Treatment with 5 micromolar MOTS-c was associated with reduced basal respiration while maintaining relatively high maximal respiratory capacity and increased extracellular acidification. This pattern may indicate metabolic reprogramming involving reduced dependence on basal oxidative phosphorylation and increased contribution of non-mitochondrial or glycolytic processes. In contrast, 10 micromolar MOTS-c resulted in a broader reduction of basal and maximal respiration as well as extracellular acidification, suggesting stronger suppression of cellular metabolic activity.

These findings demonstrate that MOTS-c modulates the bioenergetic response of astrocytes following oxygen-glucose deprivation in a dose and time dependent manner. The effects do not represent a simple increase or decrease in mitochondrial activity but rather suggest differential metabolic adaptation depending on peptide concentration and time after injury. Further studies are required to determine whether the observed increase in extracellular acidification reflects enhanced glycolytic flux.

Supported by Mossakowski Medical Research Institute, Polish Academy of Sciences, grant: FBW-058-mgr Michał Frańczak.

I-05.

Ghrelin as a Potential Regulator of Glial Activity in an in vitro Rat Model of Perinatal Asphyxia

Gargas J[1]., Ziemka-Nalecz M[1]., Sulejczak D[2]., Sypecka J[1].

[1]NeuroRepair Department, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Department of Experimental Pharmacology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: cytokines, glial cells, oxygen-glucose deprivation

Hypoxic-ischemic (HI) injury remains one of the leading causes of neurological sequelae and death among newborns. Therapeutic hypothermia remains the only available treatment; however, it is not always effective and carries a risk of complications. One of the key issues following HI is microglial activation and persistent inflammation. A growing body of research points to the properties of ghrelin, an orexigenic hormone, which reduces inflammation and oxidative stress by inhibiting microglial activation and stimulating other glial cells to release anti-inflammatory cytokines. To address this need, a study was conducted to determine the effect of ghrelin on glial cells and to assess the secretory activity of glial cells in an in vitro rat model of perinatal asphyxia. Both cerebral hemispheres were isolated from 1-day-old Wistar rats to establish a mixed glial culture. After 11 days, a microglia fraction and oligodendrocyte progenitor cells (OPCs) were isolated. The cells were cultured as single fractions or in direct co-culture of OPCs with microglia. Subsequently, some cells was subjected to an oxygen-glucose deprivation procedure, which mimics HI injury under in vitro conditions. After 24 hours, the cells and culture media were harvested and frozen for further analysis (ELISA kits, Luminex). A subset of the cells underwent immunocytochemical analysis to confirm the presence of the ghrelin receptor. Future studies will determine the effect of ghrelin on the secretory activity of glial cells. The studies confirmed the expression of the ghrelin receptor in the microglia and OPCs. Furthermore, the expression of CXCR2, CXCR4, and CXCL12 in glial cells was demonstrated, as well as the presence of cytokines such as CXCL1, CXCL10, IL-1β, IL-4, IL-6, and IL-10 in culture media. The analyses demonstrated that different types of glial cells exhibit varying levels of cytokine expression and distinct secretory profiles. Interestingly, the expression levels of certain cytokines are significantly lower in co-cultures than in individual monofractions. Interactions between glial cells may have a significant impact on the initiation of endogenous repair processes in damaged nervous tissue.

Supported by the National Science Center, grant number 2023/49/N/NZ3/02484, and Statutory grant no.6 (MMRI PAS).

I-06.

Prenatal Exposure to Valproic Acid Is Associated with Cerebellar Astrocytic Dysregulation in Young Adult Male Rats: Relevance to Autism Spectrum Disorders

Gassowska-Dobrowolska M[1]., Babiec L[1,2]., Dabrowska-Bouta B[3]., Kolasa A., Sulkowski G[3]., Struzynska L[3]., Adamczyk A[1].

[1]Department of Cellular Signalling, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Center for Social and Affective Neuroscience, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden
[3]Laboratory of Pathoneurochemistry, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[4]Department of Histology and Embryology, Pomeranian Medical University, Szczecin, Poland

Keyword: Autism spectrum disorders, astrocytes, cerebellum

The cerebellum is increasingly recognised as an important region implicated in autism spectrum disorder (ASD), extending beyond its classical role in motor coordination to cognitive, social, and affective processes. Astrocytes maintain extracellular glutamate–glutamine homeostasis and regulate the neurovascular unit through their perivascular end-feet. Although prenatal valproic acid (VPA) exposure is widely used as a model of ASD, its effects on cerebellar astrocyte-associated functions remain insufficiently characterised. This study investigated cerebellar astrocytic markers, glutamate–glutamine homeostasis, and the perivascular astrocytic compartment following prenatal VPA exposure.

Male rat offspring prenatally exposed to VPA and corresponding control animals were analysed. GFAP and aquaporin-4 (AQP4) immunoreactivity were evaluated by immunohistochemistry and confocal microscopy, with quantitative assessment of immunoreactive area and signal intensity, including ROI-based analysis. AQP4 protein abundance was additionally assessed by Western blotting. Perivascular astrocytic structures were evaluated using haematoxylin and eosin staining followed by morphometric analysis. Glutamate–glutamine homeostasis was assessed by measuring glutamine synthetase (GS) enzymatic activity and protein abundance, together with the protein levels of the astrocytic glutamate transporters GLAST and GLT1 and the amino acid transporter SN1.

Prenatal VPA exposure was associated with reduced GFAP immunoreactivity in the cerebellum of rat offspring. Morphometric analysis revealed a significant reduction in the thickness of astrocytic end-feet surrounding blood vessels in VPA-exposed animals compared with controls. In addition, AQP4 mean signal intensity was significantly reduced both within the analysed tissue area and in GFAP-defined astrocytic ROIs, whereas overall AQP4 immunoreactivity remained unchanged. These alterations were accompanied by reduced GLT1, GLAST, and SN1 protein levels and decreased GS enzymatic activity. Notably, GS protein abundance remained unchanged, indicating that reduced GS activity occurred without a detectable change in relative GS protein levels.

Collectively, these findings provide evidence that prenatal VPA exposure is associated with alterations in cerebellar astrocyte-associated processes, including glutamate–glutamine homeostasis and the perivascular astrocytic compartment. Such alterations may compromise astrocytic support of neuronal and neurovascular homeostasis and may consequently contribute to disturbances in neurotransmission and blood–brain barrier integrity, potentially representing mechanisms relevant to ASD pathophysiology.

Funding: This research was supported by Theme 3 and 9.

I-07.

Cellular composition and cryopreservation modulate the Buparlisib (BKM120) response of glioblastoma multicellular spheroids

Havelkova J[1,2]., Prasilova A[2]., Peskova K[3]., Petrenko Y[1,2].

[1]Laboratory of Biomaterials and Tissue Engineering, Institute of Physiology of the CAS, v. v. i., Prague, Czechia
[2]Department of Neuroregeneration, Institute of Experimental Medicine of the CAS, v. v. i., Prague, Czechia
[3]Czech Technical University in Prague, Prague, Czechia

Keywords: glioblastoma, buparlisib, spheroid

Three-dimensional spheroid cultures are widely used as a platform for primary screening of new therapeutic approaches. Due to their relative simplicity, spheroids offer a balance between biological complexity and experimental control.

In this study, we evaluated the response of glioblastoma (GBM) spheroids to the PI3K inhibitor buparlisib (BKM120) and investigated the influence of source cell line, BKM120 chemical modulation, stromal incorporation (mesenchymal stromal cells, MSCs), and cryopreservation.

Spheroids were generated from three GBM cell lines (U87MG, U118MG, and T98G) using Biofloat™ ultra-low-attachment plates. BKM120 (0.5 to 50 µM) was applied for 24–72 h. Spheroid growth was monitored using Incucyte®SX5, metabolic activity was assessed by resazurin assay, viability by Live/Dead assay, and 3D invasion was evaluated in a Geltrex™-based assay. Bone marrow–derived MSCs were incorporated either during spheroid formation or added on top of pre-formed GBM spheroids. To monitor their distribution, MSCs were stained with PKH26 dye. Cryopreservation was performed in 10% DMSO using a controlled cooling rate of 1 °C/min.

The treatment responses were cell-line dependent; T98G spheroids exhibited the highest sensitivity to BKM120, characterised by progressive shrinkage and reduced viability. MSC incorporation altered growth dynamics and internal cell distribution within the 3D constructs. In mixed cultures, MSCs formed a compact core, whereas seeding onto pre-formed spheroids resulted in a peripheral distribution. Exposure of mono- and multicellular 3D cultures to BKM120 for 62 h resulted in growth inhibition and concentration-dependent spheroid shrinkage, with more pronounced effects in U118MG:MSC constructs. Post-treatment 3D invasion assays showed an increased invasion in BKM120-treated multicellular spheroids compared to untreated controls or mono-cultures. Cryopreservation of 3D cultures resulted in 60–70% recovery (determined by a resazurin assay), and led to a delayed (24–48 h) invasion. Independent of the cell line, the response to BKM120 did not differ between cryopreserved and non-cryopreserved spheroids.

Therefore, spheroid models provide a controllable and tunable platform for evaluating drug response in a 3D context, capturing the effects of cellular composition and experimental handling. This supports their use in primary drug screening prior to transition to more complex organoid systems.

I-08.

Evolutionary Specialization of Human Astrocytes: GPRC5B Signaling and Interlaminar Astrocyte Development

Hemmatvand K[1]., Abbas M[1]., Ciuba K[1]., Lipiec M[2]., Virmani G[1]., Piotrowska A[1]., Knapska E[2]., Pekowska A[1].

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

Keywords: interlaminar Astrocytes, GPRC5B, astrocytes divergence

Astrocytes are essential regulators of CNS homeostasis, contributing to blood-brain barrier formation and maintenance of the extracellular environment. They actively modulate synapse formation, and pruning, thereby influencing higher cognitive functions. Importantly, many astrocytic genes display species-enriched expression patterns, suggesting human-specific mechanisms of neural regulation. Astrocytes have undergone substantial evolutionary diversification in morphology, molecular organisation, and responses to physiological and pathological challenges, accompanying the increasing cellular complexity and specialization of the human brain. Interlaminar astrocytes (ILAs) represent a specialized astrocyte population originally described in the human cortex, but their molecular and physiological functions remain poorly understood. In parallel, G protein-coupled receptor class C group 5 member B (GPRC5B) is an orphan receptor highly expressed in brain and upregulated in human astrocytes compared with mouse astrocytes. Although its function remains poorly understood, it has been implicated in astrocyte differentiation, inflammatory and homeostatic signaling, and higher-order brain functions. Here, we investigated the role of GPRC5B in human iPSC-derived astrocytes (iAstrocytes) using shRNA-mediated knockdown and established an *in vivo* platform to investigate the development and organisation of ILA-like populations. For GPRC5B, knockdown effects were assessed through calcium imaging, cell growth and bulk RNA sequencing under basal and inflammatory conditions following stimulation with the TNFα+IL1α+C1q (TIC) cocktail. GPRC5B silencing increased expression of NF-κB inhibitory regulators and attenuated ATP-induced calcium responses, indicating reduced signaling competence under basal conditions. TIC exposure induced 3793 differentially expressed genes in scrambled control iAstrocytes, compared with 2323 in GPRC5B-knockdown iAstrocytes, indicating that GPRC5B silencing attenuates the global transcriptional response to inflammatory cytokines in reactive iAstrocytes. For the mouse chimera model, H2B-GFP labelled human iAstrocytes were transplanted into the immunodeficient mouse cortex. Human GFAP immunostaining confirmed successful long-term engraftment eight months after transplantation, with transplanted cells predominantly localised within cortical layer I and extending long processes toward deeper layers, resembling the characteristic morphology of ILAs. Together, these approaches investigate molecular and cellular aspects of astrocyte evolution. Comparative human–mouse studies will determine whether GPRC5B-dependent functions are conserved or diverged between human and mouse astrocytes, while chimera model will enable molecular characterisation of ILAs and investigation of species-specific astrocyte development.

I-09.

Shedding Light on Glia: Revealing Cell Dynamics Live in 3D with Holotomographic Microscopy

Janowska J[1]., Gargas J[1]., Szymanowski J[2]., Ghete D[3]., Sypecka J[1].

[1]NeuroRepair Department, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Laboratory of Advanced Microscopy Techniques, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[3]EMEA Applications Manager, Tomocube Europe GmbH

Keywords: glia, holotomography, microscopy

Recent advances in holotomographic microscopy have enabled label-free, high-resolution 3D visualization of live cells in their native state. By exploiting intrinsic variations in refractive index, this quantitative imaging modalitiy circumvents the phototoxicity and artifacts associated with traditional fluorescence labeling, enabling long-term tracking of organelle dynamics, including mitochondria and lipid droplets. In the following study we performed live imaging of microglia, astrocytes and oligodendrocytes isolated from primary rat mixed glial cultures and cultured in physiological normoxia. Overnight acquisition enabled continuous observation of microglial phagocytic activity, oligodendrocyte maturation, and astrocyte activation. Using built-in analysis tools, we measured the refractive index (RI) of distinct organelles alongside cellular volume and dry mass, revealing key insights into state transitions and morphological changes. In conclusion, holotomography represents a powerful, non-invasive tool for glial biology, offering unprecedented visualization of subtle cellular processes that were previously difficult to capture. As analysis protocols continue to evolve, we anticipate expanding this framework to a broader spectrum of samples and quantitative measurements in future research.

Funding: Institutional Statutory Grant No. 6, Mossakowski Medical Research Institute, Polish Academy of Sciences.

I-10.

PHYSIOLOGICAL OXYGEN TENSION SHAPES SENESCENCE-ASSOCIATED TRANSCRIPTION AND NEURAL PHENOTYPE IN HUMAN DORSAL FOREBRAIN ORGANOIDS

Knapinska Z., Liput M., Zychowicz M., Buzanska L.

Department of Stem Cell Bioengineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: physiological oxygen tension (physioxia; 5% O_2), human dorsal forebrain organoids, senescence

Human brain organoids provide increasingly sophisticated models for investigating human-specific mechanisms of brain development, aging, and neurological disease. However, standard culture conditions may not adequately reproduce the physiological brain microenvironment. Atmospheric oxygen tension (21% O_2) exceeds levels encountered by neural tissue in vivo, whereas physiological oxygen tension (physioxia; 5% O_2) may improve the physiological relevance of human neural models. Here, we investigated how long-term physioxia affects senescence-associated transcription and neural phenotype in human dorsal forebrain organoids.

Human dorsal forebrain organoids were cultured for 44 days under physioxic (5% O_2) or atmospheric (21% O_2) conditions and analyzed by bulk RNA sequencing. Differentially expressed genes were integrated with the CellAge database to characterize senescence-associated transcriptional changes. Gene expression patterns were assessed using differential expression analysis, hierarchical clustering, and Gene Set Enrichment Analysis (GSEA). Neural and glial phenotypes were additionally evaluated by immunofluorescence analysis of MAP2, DCX, and GFAP expression.

A total of 186 CellAge-associated genes were differentially expressed between oxygen conditions, including 62 upregulated and 124 downregulated under physioxia. Both senescence-inducing and senescence-inhibiting regulators were represented, indicating selective remodeling of senescence-associated transcription rather than a uniform shift toward either state. Consistent with this, GSEA showed no significant coordinated enrichment of senescence-inducing (NES = -1.23, FDR = 0.113) or senescence-inhibiting (NES = -0.79, FDR = 0.949) gene sets. Physioxic organoids also exhibited increased expression of neuronal markers MAP2 and DCX and reduced expression of the astrocytic marker GFAP, suggesting altered neural differentiation under physiological oxygen tension.

These findings demonstrate that oxygen tension shapes both the molecular and cellular phenotype of human neural organoids. Physioxia selectively remodels senescence-associated transcription while promoting a more neuronal phenotype, highlighting physiological oxygen conditions as an important parameter for developing human neural models that better reflect the in vivo microenvironment and for studying aging and neurological disease.

Acknowledgments: *This work was supported by National Science Center Grant 2024/55/I/NZ5/03205 and the Statutory Fund of the Mossakowski Medical Research Institute, Polish Academy of Sciences.*

I-11.

Pro-inflammatory cytokines as markers of nicotine-induced neuroimmune changes mediated by chronic exposure in rats

Kobiela J[1]., Czuba-Pakula E[1]., Kowianski P[1,2].

[1]Department of Anatomy and Neurobiology Medical, University of Gdansk, Gdansk, Poland
[2]Department of Health Sciences, Pomeranian University of Slupsk, Slupsk, Poland

Keywords**:** nicotine, cytokines, neuroinflammation

Beyond its well-known addictive properties, nicotine is a potent modulator of neuroimmune signaling by altering glial cell activity and the expression of inflammatory mediators within the central nervous system. Previous studies indicates that chronic nicotine exposure may alter microglial and astrocytic activity as well as the levels of pro- and anti-inflammatory cytokines. However, the nature of these changes depends on the duration of nicotine exposure and the brain region examined. The aim of these study was to evaluate the effects of chronic nicotine administration on the levels of selected pro-inflammatory cytokines in rat brain tissue.

The study was performed using brain homogenates collected from 14 male Wistar rats assigned to two experimental groups: control and chronic nicotine exposure. The concentrations of IL-1beta, IL-6 and TNF-alpha were determined using enzyme-linked immunosorbent assay (ELISA).

The results demonstrated a significant decrease in IL-1beta levels in chronically nicotine-treated animals compared with the control group. In addition, no significant differences in IL-6 or TNF-alpha concentrations were detected among the experimental groups. These findings indicate that chronic nicotine exposure differentially modulate the neuroimmune response by affecting individual pro-inflammatory cytokines in distinct ways.

The obtained results indicate that chronic nicotine exposure reveal different effects on the neuroimmunological response in the brain. Integrating cytokine profiling with the assessment of glial cell activity and additional neuroimmune markers will provide a more comprehensive characterization of nicotine-induced neuroimmune alterations and contribute to a better understanding of the mechanisms underlying these changes.

I-12.

Region-specific changes in microglial, astrocyte and oligodendrocyte-associated gene expression following siponimod treatment after traumatic spinal cord injury

Kuruc T[1]., Bimbova K.K[1]., Wencel P[2]., Kisucka A[1].

[1]Institute of Neurobiology, Biomedical Research Center of the Slovak Academy of Sciences, Košice, Slovakia
[2]Laboratory of Preclinical Research and Environmental Agents, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords**:** siponimod, spinal cord injury, glial cells

Traumatic spinal cord injury (SCI) triggers a complex secondary response involving neuroinflammation, microglial and astrocytic activation, and oligodendrocyte/myelin damage. Siponimod is a selective sphingosine-1-phosphate receptor modulator targeting S1PR1 and S1PR5, with established peripheral immunomodulatory effects and emerging evidence for direct actions on CNS-resident cells. We investigated the effects of siponimod on molecular responses associated with glial reactivity and oligodendrocyte/myelin preservation following experimental SCI. Adult female Wistar rats were randomly divided into following groups: intact controls, sham vehicle (SH-V), SCI vehicle (SCI-V), and SCI treated with siponimod (2 mg/kg) (SCI-SIP). Spinal cord compression was induced using an impactor that applied a 40 g weight directly onto the exposed thoracic (Th9) spinal cord for 15 min. Animals were euthanized 7 days after surgery. Gene expression was analyzed by qRT-PCR in tissue adjacent to the lesion (+1 and −1) and at the lesion epicenter (0). Siponimod treatment was associated with a consistent reduction of S1PR1 expression across all analyzed spinal cord segments, whereas S1PR5 showed a more pronounced increase in tissue adjacent to the lesion. This receptor-associated response coincided with region-specific changes in microglial neuroprotective/reparative markers. In particular, the −1 segment exhibited increased expression of CD206, Arg1 and TGF-β, suggesting a shift toward a more reparative molecular environment. Importantly, this region also showed a coordinated mild increase in oligodendrocyte- and myelin-associated genes, including Olig2, CNPase, PLP1 and MBP, accompanied by increased BDNF expression. In contrast, these oligodendrocyte-associated changes were not observed as a coherent response within the lesion epicenter. Moreover, siponimod treatment was associated with reduced mRNA expression of activated astrocyte markers GFAP and S100B within the lesion epicenter. Overall, the findings indicate that siponimod could induce a spatially heterogeneous molecular response after SCI, with the most coordinated changes occurring in tissue adjacent to rather than within the lesion core. The concomitant modulation of S1PR1/S1PR5 expression, microglial markers associated with reparative responses and oligodendrocyte/myelin-associated genes raises the possibility that siponimod could influence tissue-reparative processes after SCI.

Supported by PAS-SAS-2024-12 a VEGA 2/0115/24.

I-13.

Gut-derived inflammatory signalling to neuronal cells in a human microfluidic gut-brain-axis-on-chip platform.

Maciejewska M[1]., Staniak K[1]., Sosna K[1]., Filipiak M[1]., Drozd M[1]., Miguel X[1]., Pietrzak M[1]., Tokarska K[1].

[1]Centre for Advanced Materials and Technology CEZAMAT, Warsaw University of Technology, Warsaw, Poland
[2]International Iberian Nanotechnology Laboratory, Braga, Portugal

Keywords: organ-on-chip, gut-brain axis, inflammatory

This study aimed to characterize gut-brain communication under peripheral inflammation, increasingly implicated in neurodegenerative disorders, using a human microfluidic gut-brain-axis-on-chip.

The PDMS platform links an intestinal and a neural chamber through microgrooves that allow soluble-factor exchange while maintaining physical separation. The intestinal compartment is collagen-coated and contains Caco-2:HT-29MTX (9:1) epithelial co-culture while neural compartment is laminin-coated and containshuman neural progenitor cells (hNPCs) differentiated on-chip in GDNF/BDNF-containing medium. Inflammation was induced by stimulation with LPS or a cytokine cocktail (TNF-α, IL-1β, IFN-γ) at different concentrations, after which both compartments were analysed by immunostaining, ELISA and western blotting

Barrier formation by the epithelial co-culture was first assessed in Transwell cultures by immunostaining,transepithelial electrical resistance (TEER) and 4 kDa FITC-dextran permeability. The co-culture formed a mature ZO-1-positive barrier confirmed by immunostaining for MUC2, villin, ZO-1 and E-cadherin with TEER rising from 28,25 to 1527,75 Ω·cm² over 20 days. Following 24 h inflammatory stimulation, TEER decreased to 73,7% and 60,4% of baseline under low- and high-dose cytokine conditions, respectively, accompanied by disrupted ZO-1 continuity.

After on-chip differentiation hNPCs identity and maturation were confirmed by immunostaining (βIII-tubulin, MAP2, NeuN). Peripheral inflammation stimulation, preliminary western blotting and immunostaining of epithelial (E-cadherin, villin, ZO-1) and neural (MAP-2, GFAP, Nestin) markers confirmed disruption of the intestinal epithelial barrier and changes in neural cell morphology, expression and distribution of neural markers.

Additionally, ELISA showed a marked rise in intestinal IL-6 and MCP-1 after cytokine exposure, reaching approximately 11- and 7-fold the levels measured in untreated cells, respectively. The neural compartment responded with a strong increase in IL-6 (approximately 30- fold) and smaller rise in MCP-1 (approximately 2-fold) those of untreated controls, respectively.

These findings show that intestinal inflammation alone can trigger responses in human neuronal cells grown on the same chip. This supports the platform's use for studying how gut-derived inflammatory signals may contribute to neurodegenerative disease.

ACKNOWLEDGMENT This work was supported by the National Science Centre, Poland under the SONATA 20 programme, grant no. UMO-2024/55/D/ST4/02457.

I-14.

Differentiation of Wharton's Jelly-derived human mesenchymal stem/stromal cells into neural lineage using physiological compounds

Maksymiuk W[1,2]., Gargas J[1]., Rytel M[2,3]., Sarnowska A[3]., Swiderska B[4]., Malinowska A[4]., Sypecka J[1].

[1]NeuroRepair Department, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Doctoral School of Translational Medicine, Centre of Postgraduate Medical Education, Warsaw, Poland
[3]Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[4]Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Keywords:mesenchymal stem/stromal cells, differentiation, CNS

Mesenchymal stem/stromal cells (MSCs) have been used in various medical treatments. They exert their therapeutic effects primarily through the secretion of anti-inflammatory and immunomodulatory molecules. In the treatment of neurodegenerative diseases, stem cell therapy is a promising approach, as MSCs are known to have protective properties in various brain disorders caused by pathological conditions or injuries. Nevertheless, in the central nervous system (CNS), it is glial cells that provide trophic support and play a neuroprotective role for nervous tissue under physiological conditions by supplying neurons with energy substrates, as well as neurotrophins and immunomodulatory molecules. The aim of the present study is to develop an effective protocol for differentiating MSCs derived from Wharton's Jelly of the umbilical cord (WJ-MSCs) into glial cells for use in the treatment of selected CNS disorders. WJ-MSCs were isolated from human umbilical cords within 24 hours after caesarean delivery. After migrating out of tissue biopsies, they were subjected to experimental protocols aimed at triggering their neural commitment. Accordingly, 24 hours after seeding, the cells were cultured in serum-free media supplemented with factors physiologically involved in neurodevelopmental processes, such as PDGF-AA, which regulates the biology of oligodendrocyte progenitors, and T3, which is involved in glial maturation. The neural commitment was verified in terms of their ability to proliferate and differentiate under the applied conditions by means of molecular biology, immunocytochemical methods and proteomics. The expression of only some of the proteins remained unchanged following differentiation. Decreased quantity of proteins involved in DNA replication, mitotic progression, confirmed reduced proliferative activity of differentiated cells. Shift in cellular priorities was also confirmed in cells as a result of the differentiated cell culture protocol. Simultaneously, the cells subjected to conditions that promote gliogenesis were characterised by changes in PDGFRα, NANOG and NG2 gene expression. The efficiency of cell differentiation was compared between different donors. Results indicate that WJ-MSCs have the potential to undergo neural differentiation and will be used to develop protocols for their efficient derivation that could potentially be used in damaged nervous tissue repairement.

Acknowledgements: Supported by National Science Center (NCN) in Poland, grant: 2022/47/O/NZ4/01161.

I-15.

The Divergent Effects of HDAC Inhibitors: Sodium Butyrate and Givinostat on Microglial Polarization Following Oxygen-Glucose Deprivation

Pawelec P., Ziabska-Paczes K., Ziemka-Nalecz M., Zajac H

NeuroRepair Department, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: givinostat, sodium butyrate, oxygen-glucose deprivation

Microglia play a central role in the neuroinflammatory response following cerebral ischemia, while also contributing to tissue repair. Their functional plasticity allows transition between pro-inflammatory and anti-inflammatory phenotypes, making microglial modulation a potential therapeutic strategy. Histone deacetylase inhibitors (HDACis) have been reported to exert neuroprotective and anti-inflammatory effects; however, their impact on microglial responses may vary depending on the inhibitor.

This study aimed to compare the effects of sodium butyrate (SB) and Givinostat (Gv) on microglial polarization and selected intracellular signaling pathways under ischemic-like conditions in vitro.

BV2 microglial cells were subjected to 40 min of oxygen-glucose deprivation (OGD) and subsequently treated with SB (1 mM) or Gv (1 μM). Cells were analyzed 24 and 72 h after OGD. The expression of pro-inflammatory (IL-1β, TNF-α, CD86) and anti-inflammatory (IL-4, arginase-1, CD206) markers was assessed by qPCR, while microglial polarization was evaluated by flow cytometry. AKT and ERK signaling were analyzed by Western blot and ELISA.

OGD markedly increased the expression of pro-inflammatory markers, particularly 24 h after injury. SB significantly reduced IL-1β, TNF-α, and CD86 expression following OGD and increased IL-4 and CD206 expression at 24 h. In contrast, Gv failed to attenuate the inflammatory response and increased IL-1β and TNF-α expression after OGD. Flow cytometric analysis revealed no statistically significant changes in the proportions of $CD11b^+CD86^+$ or $CD11b^+CD206^+$ cell populations; however, SB treatment showed a trend toward an increase of anti-inflammatory $CD11b^+CD206^+$ cell population. Notably, SB significantly enhanced AKT phosphorylation at both time points, whereas Gv did not produce a comparable sustained effect. Neither inhibitor induced consistent changes in ERK phosphorylation.

Overall, SB and Gv exerted distinct effects on microglial responses to ischemic-like injury. SB suppressed pro-inflammatory gene expression and enhanced selected anti-inflammatory markers, accompanied by increased AKT phosphorylation. In contrast, Gv did not reproduce these effects under the experimental conditions used. These findings suggest that SB may promote a shift of microglia polarization toward anti-inflammatory M2 phenotype under hypoxic–ischemic conditions, which may be associated with modulation of PI3K/AKT signaling.

I-16.

Assessment of Dexamethasone Treatment Response in Low Grade Glioma Associated Edema Using APT CEST and Diffusion Weighted Imaging A Case Study

Pietrucha Z[1]., Kulesza M[2]., Rola R[2]., Bogorodzki P[1]., Lipinski K[1].

[1]Faculty of Electronics and Information Technology, Warsaw University of Technology, Warsaw, Poland
[2]Department of Neurology, Military Institute of Aviation Medicine, Warsaw, Poland

Keywords: APT CEST, peritumoral edema, dexamethasone

Peritumoral cerebral edema significantly contributes to morbidity in patients with glioma progression. Corticosteroids like dexamethasone reduce edema by restoring blood brain barrier integrity through the upregulation of endothelial tight junction proteins. However, standard anatomical magnetic resonance imaging (MRI) provides limited insight into immediate biochemical and microstructural changes. This study evaluated short-term peritumoral tissue responses following intravenous dexamethasone administration using amide proton transfer chemical exchange saturation transfer (APT CEST) and advanced diffusion-weighted imaging (DWI).

A 43-year-old male, initially diagnosed in 2018 with an IDH-mutant grade II glioma (proliferation index 5 percent) and no comorbidities, presented in with symptomatic vasogenic edema caused by tumor regrowth. The patient was treated with 24 mg intravenous dexamethasone. MRI was performed on a 3 Tesla scanner before treatment and post-treatment. DWI was acquired with 60 gradient directions at a b-value of 1000 s/mm2. Processing included Marchenko-Pastur principal component analysis denoising and Gibbs ringing artifact removal before parameter map calculation in MRtrix. APT CEST imaging comprised six offset frequencies with WASSR B0 inhomogeneity correction, generating asymmetry maps using MolFus. Edema was semi-automatically segmented on CEST images, and masks were transferred to diffusion maps.

Following dexamethasone administration APT CEST imaging revealed a substantial shift in peritumoral tissue magnetization transfer ratio asymmetry(MTR asym), changing from -0.89 to -0.46. In contrast, diffusion metrics exhibited minimal immediate alteration: radial diffusivity changed from 7.47E-4 to 7.54E-4, mean diffusivity from 8.70E-4 to 8.50E-4, and fractional anisotropy from 0.25 to 0.27.

In conclusion, short-term dexamethasone therapy induces rapid changes in APT CEST signal within tumor-associated edema, whereas structural diffusion parameters remain largely stable over the same timeframe. The shift in MTR asym likely reflects acute changes in mobile protein-peptide concentrations and extracellular pH secondary to reduced vascular permeability. APT CEST MRI appears highly sensitive to early biochemical shifts accompanying edema resolution in IDH-mutant glioma, serving as a promising quantitative biomarker for monitoring therapeutic response in neuro-oncology.

I-17.

Development of a high throughput platform for studying nucleocytoplasmic transport defects in neurodegenerative diseases

Piorkowska G., Wrobel L.

Laboratory of Cellular Proteostasis, International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland

Keywords: nucleocytoplasmic transport, neurodegenerative diseases, high throughput platform

Ageing is the primary risk factor for most neurodegenerative diseases (NDs) and is characterised by a progressive loss in proteostasis. Defects in nucleocytoplasmic transport (NCT), which enables communication between the nucleus and the cytosol, are a hallmark of ageing and of many NDs and are hypothesized to contribute to early neuronal decline. Dysfunction of NCT is a recurring feature of NDs: TDP-43 and FUS mislocalisation is a hallmark of amyotrophic lateral sclerosis (ALS), while polyQ-expanded huntingtin disrupts nuclear pore complex function in Huntington's disease (HD). Yet how NDs-associated mutations perturb NCT remains poorly understood, and a systematic approach linking such mutations to specific NCT defects is lacking.

To address this gap, we are developing a high throughput experimental platform to systematically study nucleocytoplasmic communication in neuronal cells. We generated and validated neuronal cell lines with two types of fluorescent NCT reporters. Both reporters reliably detect disrupted NCT, capturing impairments in both nuclear export and nuclear import. We will apply them in CRISPR/Cas9-based focused screens to systematically link NDs-associated mutations to NCT defects.

This unpublished approach will help to clarify whether and how NDs-associated mutations affect nucleocytoplasmic communication and how these can drive proteostasis collapse widely observed in neurodegeneration.

I-18.

Mitochondrial DNA heteroplasmy dynamics in human cybrids: insights into selection and intermolecular recombination

Piotrowska-Nowak A.

Faculty of Biology, Insitute of Genetics and Biotechnology, University of Warsaw, Warsaw, Poland

Keywords: mtDNA heteroplasmy, mitochondrial DNA selection, intermolecular recombination

Introduction
Mitochondrial DNA (mtDNA) mutations cause a wide range of mitochondrial disorders, frequently affecting tissues with high energy demands, particularly the nervous system. Most pathogenic mtDNA variants are heteroplasmic (i.e., coexist with wild-type mtDNA molecules), and their proportions may vary between tissues and change over time, influencing disease onset, severity, and progression. However, the mechanisms governing mtDNA segregation and selection remain poorly understood. Intermolecular recombination may also contribute to mitochondrial genome diversity and stability, although its occurrence in human cells remains controversial. Understanding these processes is essential for improving the diagnosis, treatment, and prevention of mitochondrial diseases. This study aimed to develop a human cellular model to investigate mtDNA selection and assess the occurrence of intermolecular recombination.

Methods
Heteroplasmic transmitochondrial cytoplasmic hybrids (cybrids) were generated by fusing mtDNA-depleted 143B.TK- cells with mixtures of cytoplasts carrying two distinct mtDNA haplotypes differing by 10-50 nucleotides. Heteroplasmy presence and dynamics were monitored across successive cell passages using Sanger and Illumina sequencing. Native mtDNA was enriched by alkaline lysis and exonuclease V digestion and directly analyzed using long-read Nanopore sequencing to identify potential recombinant molecules.

Results
Heteroplasmic human cybrid lines were successfully established. All lines showed directional mtDNA selection toward homoplasmy, although the rate of heteroplasmy change varied between lines and over time. While some lines rapidly approached homoplasmy, others maintained heteroplasmy for multiple passages, with average changes of 0.46-0.76 percentage points per day. Selection appeared to affect entire mtDNA haplotypes rather than individual nucleotide variants. Nanopore sequencing identified very rare reads containing sequence patterns characteristic of both haplotypes, consistent with potential intermolecular recombination.

Conclusions
The established heteroplasmic cybrid model provides a valuable tool for investigating mtDNA segregation, selection, and potential recombination in human cells. These findings demonstrate genome-wide, haplotype-level mtDNA selection and suggest that intermolecular recombination may occur at very low frequency in human cells in vitro under standard growth conditions, although further validation is required. Further research into these mechanisms may improve our understanding of mitochondrial disease pathogenesis and contribute to more accurate diagnosis, disease progression prediction, and the development of therapeutic and preventive strategies.

I-19.

Region-specific astrocyte responses to oxygen-glucose deprivation contribute to differential hippocampal vulnerability to ischemic injury

Ryngwelska I., Beresewicz-Haller M.

Molecular Biology Unit, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: hippocampal regional vulnerability, endogenous neuroprotection, mitochondrial resilience

Stroke remains a major cause of death and long-term disability worldwide, and there is an urgent need for effective neuroprotective strategies beyond acute reperfusion therapies. Endogenous neuroprotection, the brain's intrinsic ability to resist injury, may provide new opportunities to improve neuronal survival following ischemia.

The hippocampus serves as an excellent model for studying endogenous neuroprotection due to its region-specific vulnerability to ischemic injury. Following transient global ischemia, the CA2, CA3, CA4, and dentate gyrus (DG) regions (CA2–4, DG) are relatively resistant, whereas the adjacent CA1 region is highly susceptible. These differences are not due to variation in blood flow but appear to result from intrinsic cellular properties. Although this phenomenon has been attributed primarily to neuronal mechanisms, accumulating evidence suggests that astrocytes may also contribute to region-specific outcomes after ischemia-reperfusion injury. We therefore hypothesized that astrocytes derived from different hippocampal regions differ in their metabolic properties and, consequently in their susceptibility to ischemic stress. To test this hypothesis, we established primary astrocyte cultures from microdissected CA1 and CA2–4, DG regions of the rat hippocampus. Under control conditions, CA2–4 and DG astrocytes showed higher metabolic activity and mitochondrial membrane potential than CA1 astrocytes, indicating greater mitochondrial capacity and metabolic resilience. Following 3h of oxygen–glucose deprivation (OGD) and 24h reoxygenation, mitochondrial activity decreased in CA1 but remained unchanged in CA2–4, DG astrocytes. Prolonged OGD progressively reduced mitochondrial activity in both populations, diminishing the regional difference. Based on these findings, the 3h OGD/R paradigm, showing the clearest region-specific differences, was selected for further analyses. Under these conditions, CA1 astrocytes exhibited decreased mitochondrial membrane potential and increased mitochondrial reactive oxygen species production, whereas CA2–4, DG astrocytes preserved mitochondrial function and exhibited higher maximal respiratory capacity. Together, these findings indicate that CA2–4 and DG astrocytes exhibit distinct mitochondrial phenotypes and greater resistance to OGD than astrocytes derived from CA1 region.

I-20.

Machine learning and deep learning (ML/DL) algorithms are sensitive, accurate, and effective tools in molecular neuroscience and clinical practice

Sokolowska B.

Bioinformatics Laboratory, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: molecular neuroscience, machine learning and deep learning methods, biomedical modeling

The research of John Hopfield and Geoffrey Hinton laid the foundation for the development of learning algorithms and AI. Their achievements have found applications in many fields of bioscience and medical technology, particularly molecular neuroscience. In 2024 (October 8), these researchers received the Nobel Prize (NP) in Physics (the first NP in history awarded for AI). That same year (October 9), the NP in Chemistry was awarded to scientists who developed the AlphaFold2 bioinformatics system, which predicts protein spatial structure using artificial neural networks (deep learning). The AlphaFold programs were based on vast resources of data and knowledge accumulated over decades (the second NP for AI). Research demonstrates the high accuracy, sensitivity, and specificity of novel bioinformatics tools.
Own experimental and biomodeling studies utilizing such modern algorithmic approaches are presented, e.g., to understand the molecular mechanisms of DNA repair and assess their effectiveness under pathophysiological conditions. Currently, ML/DL have become key tools in cell biology, neuroimaging, sequencing, and "-omics" (genomics/proteomics/metabolomics). ML/DL/AI are used to: (a) segment, track, classify, and predict cellular behavior; (b) model more complex, multiscale behaviors/structures of cells, tissues, systems, or organisms; and (c) inspiring/supporting research on neurological disorders by proposing new pharmacotherapeutic strategies and more effective methods of rehabilitation and prevention.
In conclusion, ML/DL-based techniques are already widely used in big-data-driven bioscience, digital neuroscience, and digital biomedicine. The enormous amount of collected data exceeds human capabilities in terms of classical processing, statistical analysis, and the discovery of relevant information.

I-21.

Digital and virtual biomarkers as a novel and promising approach to the (neuro)diagnosis and (neuro)therapy of neurological disorders

Sokolowska B[1]., Sokolowska E[2]., Kasperowicz S[3]., Poznanski J[3]., Maciejewska A.M[3]., Sulejczak D[1].

[1]Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2] The University of Economics and Human Sciences, Warsaw, Poland
[3]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Keywords: biomarkers, digital and virtual tools, molecular modeling

Modern technologies and AI techniques are rapidly transforming neuroscience and medical practice. Bioinformatics methods, such as virtual simulations and dynamic molecular modeling, show particular promise. Research employing these approaches is opening new pathways for advanced neurodiagnostics, neuropsychotherapy, and neuropharmacotherapy for neurological diseases, including digital neuroscience/neuropsychology and digital health/medicine. Patients can now be monitored and treated in ways previously unavailable in medical facilities - for example, through the use of the Internet of Things, information and communication technologies, nanotechnology, digital suits and sensors, extended reality environments, or machine learning and deep learning (ML/DL) algorithms.

In our report, we present examples of new digital and virtual biomarkers and our own molecular protein models, which hold tremendous potential for clinical applications in neurological diseases. These include conditions resulting from CNS injuries, as well as those of genetic origin or unknown etiology, such as autism spectrum disorders, attention-deficit/hyperactivity disorder, neuroinflammatory diseases, and neurodegenerative diseases.

In summary, technological advances at the macro, micro, and nano scales, along with big data science that leverages real-time physiological and behavioral data, are driving the development of user-friendly, effective tools for medical staff, patients, and caregivers. These tools serve as innovative or complementary diagnostic resources, often earlier and more accurate, and facilitate the monitoring and management of treatment. AI, ML, and DL models can further enhance decision-making in (neuro)diagnosis and (neuro)therapeutic procedures. Moreover, integrating conventional methods with novel digital approaches in biomarker research could revolutionize future medical practice.

I-22.

Region-specific effects of long-term metformin administration on neurochemical and structural markers in the aging rat brain

Sulejczak M[1]., Nowicka M[1]., Wieczorek M[1]., Wełniak-Kaminska M[2]., Wieteska M[2]., Stanaszek L[3]., Słupecka-Ziemilska M[1].

[1]Department of Human Epigenetics, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Magnetic Resonance Laboratory, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[3]NeuroRepair Department, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: metformin, healthy aging, brain aging

Aging is associated with progressive changes in central nervous system (CNS) structure and function. Metformin, a widely used antidiabetic drug, has recently attracted attention as a potential modulator of aging and healthspan. However, the long-term effects of metformin on the aging CNS remain poorly understood.

The aim of this study was to investigate the effects of long-term metformin administration initiated in early adulthood on selected markers of brain aging. The study was conducted in Sprague-Dawley rats receiving long-term metformin treatment (225 mg/kg body weight) from 3 months of age until the second year of life. The hippocampus and the cortical region corresponding to the prefrontal cortex were evaluated in control and metformin-treated (MET) animals. Magnetic resonance spectroscopy was used to assess differences in the concentrations of selected metabolites, while immunohistochemical analyses were performed to evaluate astrocyte and axonal coverage.

Long-term metformin administration was associated with a higher concentration of glutathione and a lower concentration of the glutamate/glutamine complex in the cortical region corresponding to the prefrontal cortex. In the hippocampus, metformin treatment was associated with a higher ~1.7 ppm macromolecular signal. Immunohistochemical analysis revealed lower axonal coverage in the CA1 region and a higher number of nuclei in the dentate gyrus in the MET group.

At this stage of the study, the results suggest that long-term metformin administration from early adulthood may be associated with region-specific neurochemical and structural changes in the aging brain. Further studies are planned to elucidate the underlying mechanisms and determine the potential implications of these changes for brain aging.

I-23.

SorCS2 shapes astrocyte secretome and astrocyte-to-myeloid cell signaling after ischemic stroke

Tempes A[1]., Klachacz A[1]., Bakun M[2]., Czerny K[1]., Piatek S[1,3]., Koschutzke L.T[3]., Daugardt A[3]., Jariwala V.D[4]., Joseph K[4]., Harms C[3]., Malik A[1].

[1]Cellular Neurobiology Research Group, Faculty of Biology, University of Warsaw, Warsaw, Poland
[2] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
[3] Department of Experimental Neurology, Charité-Universitätsmedizin, Berlin, Germany
[4] Department of Neurosurgery, Medical Center - University of Freiburg, Freiburg, Germany

Keywords: astrocytes, stroke, secretome

Background: Astrocytes are key regulators of the brain microenvironment and play an important role in the response to ischemic stroke. Following ischemic injury, astrocytes become activated and undergo profound changes in their secretory activity, contributing to inflammation, glial scar formation, and extracellular matrix (ECM) remodeling. Transforming growth factor-beta (TGFβ), which is strongly upregulated after ischemic stroke, promotes astrocyte activation and induces expression of SorCS2, a VPS10P-domain receptor involved in intracellular protein sorting. While SorCS2 has been extensively studied in neurons, its role in astrocyte secretory function and post-stroke microenvironment remodeling remains poorly understood.

Objective: We investigated whether SorCS2 regulates the secretory activity of TGFβ-activated astrocytes and whether its depletion affects the post-ischemic brain microenvironment.

Methods: Primary astrocytes from wild-type and SorCS2-deficient mice were stimulated with TGFβ, and their secretomes were analyzed using mass spectrometry-based proteomics. Differentially secreted proteins were further evaluated with a focus on ECM-associated factors. The in vivo relevance of these findings was investigated using a mouse model of ischemic stroke induced by middle cerebral artery occlusion (MCAo) in mice with astrocyte-specific depletion of SorCS2, with particular attention to ECM composition and myeloid cell morphology in the affected hemisphere.

Results: TGFβ-activated astrocytes secreted multiple proteins associated with ECM organization and remodeling. Loss of SorCS2 significantly altered the astrocyte secretome, including the release of ECM components such as biglycan. Reduced secretion of biglycan was observed in SorCS2-deficient astrocytes, suggesting that SorCS2-dependent intracellular sorting contributes to the extracellular distribution of specific ECM proteins. Moreover, astrocyte-specific SorCS2 deficiency in the mice model of MCAo was associated with altered myeloid cell morphology in the ischemic hemisphere, indicating changes in the post-stroke brain microenvironment and inflammatory response.

Conclusions: Our findings identify SorCS2 as a regulator of astrocyte secretory function and ECM remodeling following ischemic injury. By linking intracellular protein sorting in astrocytes to changes in the extracellular environment and myeloid cell properties, this study provides new insights into mechanisms underlying astrocyte-mediated responses to stroke and highlights SorCS2 as a potential molecular target for modulating post-stroke brain repair.

I-24.

Activity of the phrenic and hypoglossal nerves in an Alzheimer's disease model under conditions of hypoxia and cholinergic system stimulation

Wrzesien A[1, 2]., Andrzejewski K[1]., Jampolska M[1]., Kaczynska K[1].

[1]Department of Respiration Physiology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Doctoral School of Translational Medicine, Centre of Postgraduate Medical Education, Warsaw, Poland

Keywords: Alzheimer's disease, respiratory nerves, cholinergic system

Alzheimer disease (AD) is neurodegenerative disorder that progresses to a gradual decline in cognitive function. AD patients expirience also breathing disturbances such as decreased respiratory muscle strength, impaired lung function, and a high prevalence of obstructive sleep apnea, which by causing hypoxia, exacerbates neurodegeneration. The aim of this study was to investigate the effect of cholinergic system activation with rivastigmine on the activity of respiratory nerves in a rat model of streptozotocin (STZ) – induced AD. Three weeks after intracerebral injection of STZ rats were anesthetized, tracheostomized, connected to a ventilator, and vagotomized. The phrenic (PHR) and hypoglossal (HG) nerves were transected and placed on bipolar electrodes for nerve activity recording. Hypoxic exposure was performed with 8% O_2 and maintained until apnea occurred. 10 minutes after the end of apnea, rivastigmine (0.3 mg/kg iv) was administered and 5 minutes after, rats were subjected to second hypoxia. Next, the hippocampi and brainstems were harvested to measure acetylcholine (ACh) levels and assess abnormalities in the cholinergic system. Under hypoxia, a significant increase in the activity of both HG and PHR nerves was observed in all rats, without difference between the study groups. Rivastigmine injection caused a significant reduction in the HG and PHR amplitudes under hypoxic conditions in the STZ group only. No significant difference was observed in the duration of hypoxia required to induce apnea among the study groups. However, a significantly longer arrest of breathing was observed in STZ animals. Treatment with rivastigmine had no effect on either the duration of hypoxia required to induce respiratory nerve inactivity or the duration of apnea in either group. STZ rats showed decreased ACh levels by 25% in the brainstem in comparison to sham rats.
Abnormality in the brainstem ACh levels of STZ-treated rats is associated with increased sensitivity of these animals to AChE inhibitor, as evidenced by reduced activity of both respiratory nerves during hypoxia. The prolonged duration of apnea following hypoxia and the altered activity of respiratory nerves after rivastigmine indicate impaired respiratory control in the STZ-induced AD model.
The study was funded by the National Science Centre 2022/45/B/00221.

I-25.

Exogenous resistin, a dementia-associated peptide, attenuates respiration and triggers asthma-like respiratory responses in mice

Zajac D., Russjan E., Kaczynska K.

Department of Respiration Physiology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: resistin, respiration, dementia

Resistin, a pro-inflammatory peptide of the hunger-satiety axis, gains increased attention in neurodegenerative diseases as its high levels are associated with dementia. It is supposed to be a metabolic link between metabolic dysfunction, where its levels are increased, and neurodegeneration and neuroinflammation in the brain. One of the risk factors of neurodegenerative disorders, including Alzheimer's disease, are not only metabolic disturbances like the metabolic syndrome or obesity but also poorly controlled respiratory diseases including asthma, in which resistin itself is elevated. Thus, the aim of the present study was to evaluate whether artificially elevated resistin levels would affect respiration and symptoms of non-atopic asthma, a severe form of asthma, often linked to obesity.

The DNFB/DNS non-atopic asthma model has been used. Male Balb/c mice received i.p. resistin during the entire sensitization period and underwent afterwards plethysmographic measurements of respiratory parameters followed by cytological and biochemical analyzes of lung homogenates and broncho-alveolar fluid. Control mice received saline.

Resistin administration decreased minute ventilation in both healthy and asthmatic mice, mostly at the level of tidal volume by up to 30%. At the same time, resistin induced in healthy animals airway hyperreactivity comparable to the one observed in asthmatic animals which correlated with inflammatory markers like cellular influx into the airways. Additionally, resistin administration in asthmatic mice lowered these responses slightly alleviating airway hyperreactivity.

In conclusion, resistin impacts respiration and its elevated levels as observed in several forms of dementia, contribute to inflammation of the respiratory tract of prone subjects.

I-26.

The Effect of an MSC-Derived Product ABX-0707 on the Primary Cortical Neurons and their Co-culture with Glial Cells in an In Vitro Rat Model of Neonatal Asphyxia

Ziabska-Paczes K[1]., Gargas J[1]., Pelczarska M[2]., Pobudkiewicz A[2]., Rytel M[2]., Ziemka-Nalecz M[1]., Sarnowska A[2]., Sypecka J[1].

[1]NeuroRepair Department, Mossakowski Medical Research Insitute, Polish Academy of Sciences, Warsaw, Poland
[2]Translational Platform for Regenerative Medicine, Mossakowski Medical Research Insitute, Polish Academy of Sciences, Warsaw, Poland

Keywords: perinatal asphyxia, glial cells, stem cells

Perinatal hypoxic-ischemic brain injury, known as perinatal asphyxia, is one of the main causes of permanent brain damage that can lead to long-term neurological disorders and, in extreme cases, to neonatal death. Despite numerous studies, therapeutic hypothermia remains the only neuroprotective method used in newborns with perinatal asphyxia. However, the use of hypothermia is not always possible. Among new therapeutic strategies, biological agents such as stem cells are gaining popularity. To address the increasing demand for new therapies, this study used an in vitro model of perinatal asphyxia, involving primary cortical neurons and co-cultures of primary cortical neurons with glial cells. Cells were isolated from the brains of 1-day-old Wistar rats. Since the cells that form the brain's neural tissue are interdependent, it was decided to simulate in vivo conditions by creating a co-culture model of neurons with oligodendrocyte progenitor cells (OPCs), which support neuronal function, are responsible for myelination, and are also extremely sensitive to hypoxic-ischemic injury. Cells were cultured under physiological normoxic conditions (5% O_2, 5% CO_2); subsequently, a part of the culture was exposed to hypoxic-ischemic conditions by removing glucose and oxygen from the culture medium (OGD procedure) for 40 minutes, effectively imitating hypoxia-ischemia under in vitro conditions. Following this procedure, cells were cultured in fresh medium supplemented with a mesenchymal stem cell (MSC) derived product called ABX-0707 at various concentrations. The biological material collected after 24 hours was then subjected to additional analyses, such as cytotoxicity evaluation using the LDH assay, cell viability assessment using the Sytox dye, and cell proliferation assessment using the anti-Ki67 marker. A 24-hour live cell observation was also conducted using the Tomocube HT-X1 holotomography system. Based on the results obtained, it was demonstrated that the ABX-0707 product is not cytotoxic to primary cortical neurons or to co-cultures of neurons and glial cells, and that in groups subjected to the OGD procedure, it exerts a neuroprotective effect and increases cell proliferation with a noticeable dose-dependent effect.

Funding: Medical Research Agency as part of the National Reconstruction Plan.

I-27.

Oxygen-dependent metabolite profiles in human embryoid bodies following methylmercury chloride exposur

Zieminska E[1]., Augustyniak J[1]., Toczylowska B[2]., Salinska E[1].

[1]Department of Neurochemistry, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
[2]Department IV Laboratory of Supporting Diagnostics and Therapy of Metabolic Diseases, Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, Warsaw, Poland

Keywords: embryoid bodies, methylmercury chloride, NMR metabolomics

Embryoid bodies (EBs) derived from human induced pluripotent stem cells (hiPSCs) resemble early stages of human embryonic development and provide a useful in vitro model for developmental toxicity studies. EBs exposed to methylmercuric chloride (MeHgCl) under different oxygen conditions exhibited oxygen-dependent differences in genome integrity, DNA repair, mitophagy, and differentiation. In the present study, we investigated whether oxygen concentration modulates the profiles of hydrophilic and hydrophobic metabolites in EBs after chronic exposure to low doses (1 nM) of environmentally relevant MeHgCl. EBs were cultured in 5% O_2 or 21% O_2 in the presence or absence of MeHgCl. Metabolic profiles were obtained from the NMR spectra of EBs. Statistical analysis of the results was performed using uni- and multivariate tests. Oxygen-dependent differences were demonstrated in baseline metabolite profiles (control group) and in the metabolic response to MeHgCl (test group). The oxygen-dependent metabolic differences involved metabolites related to amino acid metabolism, nucleotide-related compounds, phospholipid-related signals, and fatty acid-related fractions. EBs cultured under 5% O_2 displayed a more regulated and adaptive metabolic response to MeHgCl, whereas 21% O_2 was associated with oxidative imbalance and more pronounced metabolic remodeling. Overall, oxygen concentration emerged as a key factor shaping the metabolic response to MeHgCl exposure. These findings extend previous observations on oxygen-dependent MeHgCl toxicity in EBs and support the use of oxygen-controlled human stem cell-derived models for realistic assessment of the developmental hazards associated with environmentally relevant methylmercury exposure.

I-28.

A patient-derived iPSC neuroorganoid platform for modeling Dravet Syndrome, schizophrenia and Huntington's Disease for preclinical therapeutic testing

Zychowicz M., Kuczynska Z., Metin E., Lech W., Czeredys M., Buzanska L.

Department of Stem Cell Bioengineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Keywords: brain organoids, patient-derived iPSC, neurological diseases

Neurological and neuropsychiatric disorders remain difficult to study and treat, partly because conventional animal and 2D cell models do not fully recapitulate human-specific and patient-specific disease mechanisms. Here we present a platform of human neuroorganoids derived from induced pluripotent stem cells (iPSCs) of patients with Dravet syndrome, schizophrenia and Huntington's disease (HD), together with matched controls. We developed standard operating procedures (SOPs) for the generation of region-specific organoids: ventral forebrain organoids for Dravet syndrome, dorsal forebrain organoids for schizophrenia, and striatal organoids for HD. The organoids are characterized using a multi-level approach that includes viability assays, phenotypic and cell-type characterization, functional assessment of neuronal network activity by microelectrode array (MEA) recordings, metabolic profiling, and single-cell RNA sequencing (scRNA-seq) to resolve cellular composition and transcriptional profiles. In addition, we established a cryobanking workflow for organoids, enabling long-term storage and on-demand availability of standardized material.

Taken together, this workflow provides a foundation for a scalable, reproducible platform. It is designed for preclinical evaluation of candidate therapeutic compounds, including efficacy and toxicity screening, and offers a human-based, patient-specific alternative that can support research on neurological diseases and reduce reliance on animal models.

This work was funded by the National Recovery and Resilience Plan (Poland), implemented by the Medical Research Agency grant KPOD.07.07-IW.07 0105/24