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ABSTRACT BOOK

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Doskonała Nauka

Opening lecture

Fast, Automated, and Integrated: Electrochemical Sensors and Algorithms Transforming Blood and Bioliquids Analysis

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Electrochemical sensors have been central to both research and clinical diagnostics for decades. Their widespread use in clinical diagnostics - particularly in measuring electrolytes and metabolites in body fluids such as blood, urine, and sweat - is driven by high selectivity, fast response times, durability, and cost-effectiveness. The ability to deliver rapid and accurate electrolyte measurements has made the sensors indispensable tools in emergency diagnostics, critical care, and health monitoring.
With experience bridging academia and industry, the author has encountered numerous challenges related to the research, development, and implementation of the sensors in clinical chemistry. The success of sensor technology hinges on two critical factors: (1) innovative design capable of accurate measurements in large sample numbers and small sample volumes, and (2) rapid, reliable reporting of results. The latter is often considered proprietary, embedded within instrument software, calibration algorithms, and guided by reference materials and methods established by international recommendations, including those from the International Federation of Clinical Chemistry (IFCC) or the European Union in vitro diagnostics regulations. The lecture provides insights into that lesser-known area.
The presentation, providing real-life academic and industrial research examples, will explore and expose the pivotal role of data-driven algorithms and modeling techniques in creating integrated workflows for clinical instrumentation. Examples will trace the development of ionized magnesium analyzer from a start to a commercial product, miniaturized all-solid-state sensor platforms for remote electrolyte measurement in biofluids, and interconnected high-throughput hospital networks. It will demonstrate how intelligent data processing combined with numerical modelling enhances speed, accuracy, and reliability in clinical diagnostics, addressing the present biomedical requests, at every stage from sensor to screen.
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Inaugural lecture

Harnessing Big Data in Neuroscience: Strategic Insights into Data Sharing and Machine Learning Applications

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The rapidly increasing volume and complexity of data in neuroscience research necessitate effective solutions for organizing and managing large datasets. This is essential for enhancing data discoverability, interpretability, access (both open and restricted), and reuse. Moreover, building a critical mass of actionable data is crucial for advancing AI applications in neuroscience, allowing for the development of more sophisticated algorithms capable of deeper insights and analyses of complex datasets. To address these challenges, the EBRAINS RI, the European distributed infrastructure for brain and brain-inspired research, has developed a comprehensive suite of services: The Data Services are designed to accelerate discoveries, allowing researchers to publish citable, well-annotated datasets alongside research articles. Users can search for and access relevant datasets for comparison or reuse, all within an online environment that supports analytical tools and workflows, fostering a collaborative research culture. The Atlas Services provide digital 3D atlases of the human brain, as well as brains of mice, rats, and non-human primates. The services include tools for registering and analyzing data in the context of atlas-defined regions and coordinate spaces. Integrated with the Data Services, users can perform searches that specify data locations within the brain, either at defined brain regions or precise atlas coordinates. Spatial atlas navigation tools further enhance data discoverability and interpretability. The Analytical Tools and Workflows leverage both the Data and Atlas Services to support the development and execution of data analysis, modeling, and simulation. Combining these services with AI approaches enables researchers to conduct deeper and more efficient analyses of their data. The platform provides controlled access to collaborative workspaces, facilitating teamwork and data sharing as well as access to computing resources, including HPC environments and JupyterLab. Overall, the EBRAINS RI offers stable and robust solutions for data-driven research, empowering researchers to effectively perform reproducible and replicable neuroscience. Supported by the European Union's Research and Innovation Program Horizon Europe under Grant Agreement no. 101147319 (EBRAINS 2.0).

Plenary lectures

PL.1. Beyond dystrophin: novel targets for treatment of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked disease caused by mutations in the D*MD* gene, encoding dystrophin, an essential structural protein for skeletal muscle, the heart, and neurons. The disease is progressive, leading to loss of ambulation in boys in their early teens, and is ultimately fatal, typically in the third decade of life. Although several therapies, including genetic ones, have been approved, their effectiveness remains limited.
In our studies, we have explored the role of modulatory genes by generating double-knockout dystrophic (*mdx*) mice, lacking both dystrophin and heme oxygenase-1 (HO-1, *Hmox1*). We showed that the absence of HO-1 aggravated DMD progression. Inversely, transgenic *mdx* mice overexpressing human *HMOX1* in satellite cells, the muscle stem cells, demonstrated partial amelioration of disease symptoms. Interestingly, deletion of *miR-378* in *mdx* mice improved exercise capacity and mitigated impaired glucose tolerance and insulin sensitivity.
In parallel, we also revealed altered expression levels of hydrogen sulfide (H_2S)-producing enzymes in dystrophic muscles. Accordingly, we demonstrated that supplementation with H_2S-donors has the potential to modulate disease progression and improve the exercise capacity of dystrophic mice.
Investigating cardiac dysfunction in DMD remains challenging due to the limitations of animal models. To address this, we employed a “patient-in-a-dish” strategy, relying on human induced pluripotent stem cells (hiPSCs), hiPSC-derived cells, and 3D cardiac microtissues (“organoids”). Dystrophin-deficient hiPSC-cardiomyocytes, either patient-derived or generated *via* CRISPR/Cas9 gene editing, exhibited disrupted iron homeostasis, a phenotype also observed in hiPSC-cardiomyocytes from patients with Becker muscular dystrophy, a milder dystrophinopathy. Notably, correction of the DMD mutation *via* CRISPR/Cas9 or pharmacological modulation restored normal iron handling.
Finally, we showed that dCas9-mediated upregulation of utrophin expression, the paralogue of dystrophin, improved the properties of dystrophin-deficient cardiomyocytes, normalizing their impaired calcium handling.
Collectively, these findings highlight a range of genetic, epigenetic, and metabolic strategies with therapeutic potential for modulating DMD pathology in both skeletal and cardiac muscles.

Plenary lectures

PL.2. Bridging the Gap: Transitioning Brain Imaging Techniques from Lab to Clinic

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Over the past decade, Professor Steve Williams and colleagues at the Department of Neuroimaging, King's College London, have focused their research efforts on making neuroimaging a scalable and accessible tool. During this presentation, he will highlight some recent innovations in the development of silent, rapid, and motion-sensitive MR imaging and the creation of a new portable MRI system that is now being used globally to assess brain health in lower and middle-income country settings. The talk will also touch upon new approaches to the assessment of cognitive deficit in psychiatric and neurological disorders, where there are very few effective therapeutics that target these symptoms. A comprehensive assessment of these deficits is essential for identifying therapeutic targets in a data-driven manner. To fill this gap, we developed a set of brief online cognitive tasks, designed to be device-agnostic and repeatable, allowing for cost-effective remote assessment at scale. This battery was refined after an initial validation in healthy controls, then used to assess patients with Major Depressive Disorder or psychosis. A subset of this cognitive battery has now been adapted for administration during fMRI in order to map the neural circuits of these tasks. Initial neuroimaging results from healthy volunteers will be shared as we plan to extend these investigations into patient groups. We hope that these neuroimaging findings will provide insights into the pathophysiological mechanisms of cognitive dysfunction in psychiatric disorders. This will inform the development of targeted therapeutic interventions, which include pharmacology, neurostimulation, and cognitive training, aimed at restoring network connectivity and improving cognitive outcomes in affected individuals. Understanding these brain circuit abnormalities is crucial for advancing personalized treatments and improving the quality of life for those suffering from psychosis and major depression.

Invited Sessions

Session 1: Exploring disease and treatment associations in genome data banks

S.01-1. Ukraine's Type 1 Diabetes (T1D) Research Initiative

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This talk will outline our strategic efforts to address the global challenge of "genomic deserts," regions that lack sufficient genomic data, which hampers the development of fair and unbiased health solutions. We have established a platform that not only aggregates but also updates whole-genome data from a variety of international and national genomic projects. This platform has allowed us to analyze global trends in whole-genome sequencing and identify significant disparities in data availability. These findings underscore the critical need for an inclusive approach in genomic studies that integrates underrepresented populations. In Ukraine, where resources for genome research are limited, we have built the necessary infrastructure and cultivated local expertise in genomics to address these disparities. Our ongoing research focuses on uncovering the genetic determinants of Type 1 Diabetes (T1D), with a special emphasis on rare and local variants that are often overlooked in broader studies. Through a comprehensive genome-wide association study (GWAS), we are mapping genetic diversity and identifying novel genetic factors linked to T1D. This project has led to the development of a T1D Research Network, a local biobank, and the sequencing of 20,000 exomes, all contributing to an open-access genetic database that fosters global collaboration. Preliminary analyses highlight the significant role of HLA haplotypes and other genes, reinforcing the utility of our cohort for further research. This initiative not only enhances our understanding of T1D but also aims to create a more equitable landscape in genomic research, ultimately improving disease prevention and treatment strategies through better representation of human genetic diversity.

S.01-2. Separating direct, indirect and parent-of-origin genetic effects in the human population using genome data banks

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Background: An individual's traits result from two sources: the expression of their DNA and the environment that they experience. Parental characteristics shape a child's environment, where the genotypes of both the mother and father influence the traits of their children, beyond simply the alleles that are inherited (indirect genetic effects). Additionally, parents induce epigenetic modifications of an individual's DNA, further complicating the genotype-phenotype relationship.
Materials and Methods: We present a novel method to estimate the direct, indirect maternal and paternal, and parent-of-origin effects. Applying our model to 30,000 child-mother-father trios with phased DNA information from the Estonian Biobank (EstBB) and the Norwegian Mother, Father, Child Cohort (MoBa), we jointly estimate the phenotypic variance attributable to these four effects, unbiased of assortative mating for height, body mass index (BMI) and childhood educational test score (EA). Moreover, we use a multiple regression to identify associated DNA loci with each of the traits.
Results: For all three traits, direct effects make the largest contribution to the genetic effect variance. But we find that the combined parental indirect genetic effects are equally important, and that there is a non-zero parent-of-origin effect variance for all traits. Using the partitioned variance estimates, we calculate the heritability that would be obtained at the population level under random mating for common DNA loci. The proportional contribution of direct effects to these heritability values can be calculated as 64.0% for EA in MoBa, 77.1% and 63.4% for height in MoBa and EstBB, and 81.2% and 88.0% for BMI in MoBa and EstBB. Additionally, using within-family genome-wide association testing, we identify 276 independently associated DNA regions that replicate across two additional biobanks. All identified regions show a genotype-phenotype relationship that reflects an interplay of direct, indirect, and parent-of-origin effects.
Conclusions: Separating direct, indirect, and parent-of-origin effects in the human population is challenging, but is needed to understand the influence of parental genotypes on their children's characteristics. This requires joint modeling of parental and child genotypes alongside the parental origin of loci.

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S.01-3. Unsupervised learning of pathway activity importance in individual samples: An example from scRNA-Seq

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Pathway enrichment analysis is a key component of bioinformatics workflows. With the advancement of single-cell RNA sequencing (scRNA-Seq), single-sample pathway enrichment (PE) algorithms have gained popularity, as they enable the assessment of gene expression patterns at the level of individual cells. This allows for precise investigation of pathway-level deviations across cellular populations. However, a major challenge remains: determining the relative significance of pathway activity within individual cells. To address this, we propose an unsupervised pipeline for grouping pathway activities across the analyzed cells.
Four scRNA-seq datasets (PBMC, COVID, Bone Marrow and Liver) of known immunological cells and different volume, were collected. Gene expressions were log-normalized and transformed into pathway activity scores (PAS) using sets of genes representing signatures of immunological processes. Gene sets were extracted from publicly available databases and marked into target cell type. Transformation into PAS was performed by the six known single-sample algorithms (CERNO, AUCell, ssGSEA, Z-score, JASMINE, Mean). Moreover, we have introduced own solution based on count binarization (BINA). Next, the PBMC dataset was used to asses gensets with clear target for cell types by maximization of ARI index. Then, we propose usage of Gaussian Mixture Modeling with AIC criterion on each PAS vector. To extract relatively important pathway activity in each cell, the component with the highest mean was taken. To assess results ARI and FDR were calculated based on initial cell types and pathway target. This approach was compared to the sole existing solution based on heuristic statistic: AUCell thresholding.
Our GMM-based method consistently outperforms AUCell thresholding in terms of false discovery rate (FDR) across all tested PAS transformations. Additionally, the Adjusted Rand Index (ARI) indicates that our approach yields better performance than AUCell, CERNO, and ssGSEA algorithms. We further evaluated the method using breast cancer datasets spanning three different omics layers, with results confirming previously observed patterns.
In summary, our approach effectively identifies the relative importance of pathway activity across cells, regardless of dataset size or the specific PAS transformation applied. Notably, unlike existing methods in the field, our solution is fully unsupervised.

S.01-4. Interplay between lipid metabolism and microRNAs in the long-term effects and intergenerational transmission of childhood trauma

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Background: Childhood trauma is an important risk factor for psychiatric and physical ailments during adulthood. Emerging evidence from rodent studies suggests that some behavioral and metabolic symptoms of childhood trauma are transmissible across generations. However, the translational implications of this novel concept are in the preliminary stages.
Materials and Methods: This project involved a systematic examination of epigenetic regulators, specifically small non-coding RNAs, in serum, sperm, and milk samples collected from ethnically diverse human trauma cohorts. The overarching aim was to identify the molecular underpinnings of the long-term effects and transmission of psychological trauma symptoms. Small RNAs were analyzed in:

1. The serum of Pakistani children with recent trauma in the form of paternal loss and maternal separation (PLMS),
2. The sperm of adult Pakistani men with a history of complex trauma before age 17,
3. The milk of lactating Polish mothers with a history of adverse childhood experiences, and
4. The serum and sperm of adult men and women exposed to the Srebrenica genocide in Bosnia & Herzegovina during childhood.

Results: Pathway analysis of differentially expressed small non-coding RNAs across these samples suggested a potential role for cholesterol signaling in the long-term propagation and transmission of trauma symptoms. Notably, miR-223-3p was consistently upregulated in blood, sperm, and milk samples from trauma-exposed individuals. This microRNA targets SR-B1, the receptor for high-density lipoproteins (HDLs), implicating cholesterol biosynthesis in trauma-related epigenetic regulation.
Conclusions: These findings indicate a conserved molecular signature involving cholesterol signaling and miR-223-3p in the biological embedding and possible transmission of childhood trauma. Current efforts are focused on modeling the role of lipids and lipid-associated factors in trauma transmission using ethologically relevant mouse models and ex vivo approaches.

Session 2: Mechanisms and modeling of non-communicable diseases

S.02-1. Vascular fibroblast (dys)function as new causal mechanism in vascular ageing and hypertension

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Fibroblasts inhabit the adventitial layer of blood vessels. While fibroblasts are annotated inside atherosclerotic plaques, their identity and function are unclear. Presumably, specific fibroblast markers and in-depth heterogeneity analysis are currently lacking, hindering functional studies in cardiovascular diseases (CVD). The Sluimer lab has established new cell-type and subset markers in murine and human arteries and studied the adventitial fibroblast response to CVD and its risk factors, hypercholesterolemia, and aging. The function of a new gene marker for fibroblasts was studied in detail. SNPs in the gene correlated with blood pressure in humans and caused hypertension in aged knockout mice. Thus, fibroblasts are causally involved in vascular pathology and represent a potential new avenue for treatment.

S.02-2. Tale of two tubes exploring mechanisms of failing human heart - focus on cardiac microtissues

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Cardiovascular diseases remain a leading cause of death and disability in developed countries, with sudden cardiac death accounting for approximately 15–20% of all deaths. To better understand cardiac pathologies and screen potential therapies, 3D culture models composed of various cardiovascular cell types are increasingly used.
We developed scaffold-free, self-aggregating 3D cardiac microtissues (MTs) using human induced pluripotent stem cell (iPSC)-derived cardiomyocytes and human cardiac fibroblasts (HCFs). Fibrotic conditions were induced via treatment with the profibrotic cytokine TGF-β, fetal cardiac fibroblasts exposed to TGF-β, or fibroblasts derived from heart failure patients (HF-HCFs).
Our findings show that activated or diseased HCF regulate myocardial contraction through β-adrenoreceptor signaling. Addition of human monocyte-derived macrophages, key modulators of fibrosis, affected the fibrotic response and contractility of MTs. Specifically, healthy macrophages, but not those from diseased donors, reduced TGF-β1-induced fibrosis and improved MT function.
Proteomic analysis revealed elevated levels of dysferlin (DYSF) in HF-HCFs. DYSF emerged as a potential antifibrotic protein involved in the TGF-β–FOSL2–autophagy signaling axis. Knockdown of DYSF in HCFs worsened MT morphology, contractility, and apoptosis, indicating its protective and antifibrotic role in cardiac fibrosis.
Treatment with SD208, a TGF-β receptor type 1 inhibitor, successfully reduced fibrotic features in MTs. Similarly, PPAR agonists—elafibranor (PPARα/δ) and lanifibranor (PPARα/δ/γ) and metformin, a common antidiabetic drug with cardioprotective properties, reversed fibrotic changes and improved MTs contractility.
To explore systemic interactions, we established a multi-organ-on-a-chip platform combining cardiac and liver MTs. Remarkably, under fibrotic conditions, liver MTs mitigated TGF-β-induced fibrosis and promoted cardiac MT survival.
In conclusion, our scaffold-free 3D cardiac MT model recapitulates key features of cardiac fibrosis and provides a high-throughput, physiologically relevant system for drug testing and mechanistic studies of heart disease.

S.02-3. The human heart at single cell resolution

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Single-nucleus RNA sequencing (snRNA-seq) has become a powerful tool in molecular cardiology, enabling high-resolution profiling of gene expression at the level of individual nuclei within complex tissues. Applied to cardiac tissue, snRNA-seq allows for precise identification of diverse cellular populations, detailed characterization of transcriptional changes linked to disease progression, and discovery of novel pathogenic pathways and therapeutic targets.
In our study, we leveraged snRNA-seq to investigate molecular alterations in the right ventricle (RV) of patients with end-stage dilated cardiomyopathy (DCM). We analyzed 158'012 nuclei isolated from snap-frozen RV tissue samples obtained from 21 male DCM patients and 4 male healthy controls. To explore relationships between gene expression and clinical measures of disease severity, we integrated patients' echocardiographic and hemodynamic data, as well as age, as continuous variables within a generalized linear model framework.
In DCM samples, transcriptional alterations were most pronounced in cardiomyocytes, with the majority of differentially expressed genes (DEGs) correlating with severity of pulmonary hypertension (measured as mPAP) rather than with left or right ventricular structural or functional metrics. We identified 982 genes whose expression positively correlated with mPAP and 2215 genes that correlated negatively. Interestingly, non-coding transcripts accounted for 10.1% of upregulated genes but a striking 64.7% of downregulated genes. Gene set enrichment analysis revealed that higher mPAP levels were associated with increased expression of genes involved in cardiomyocyte contraction and remodeling, autophagy, adrenergic signaling, endosomal transport, and glucose metabolism. To identify potential biomarkers of RV dysfunction, we examined mPAP-associated DEGs in cardiomyocytes from healthy RV tissue and compared these results with published snRNA-seq datasets from DCM LV samples. After focusing on genes encoding plasma-secreted proteins, we identified 68 genes positively associated with mPAP (including FLNC, MYL9, ZYX) and 52 genes negatively associated with mPAP as promising candidates for circulating biomarkers of RV dysfunction.
In conclusion, snRNA-seq analysis provides novel insight into the molecular landscape of RV dysfunction in end-stage DCM, highlighting pulmonary hypertension severity as the principal driver of transcriptional remodeling in RV cardiomyocytes and underscores the complex cellular responses to RV pressure overload. These findings lay the groundwork for future mechanistic studies and biomarker validation.

S.02-4. Endothelial cell senescence at the crossroads of inflammation and vascular dysfunction

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Background: Endothelial cell (EC) senescence is a key contributor to vascular dysfunction and chronic inflammation, playing a central role in the progression of age-related and metabolic vascular diseases. Senescent ECs exhibit phenotypic and functional changes, including acquisition of a senescence-associated secretory phenotype (SASP), which may modulate intercellular communication and immune responses. The mechanisms by which senescent ECs influence vascular function and immune interactions remain incompletely understood.
Materials and methods: We used a replicative senescence model based on continuous passaging of human umbilical vein endothelial cells (HUVECs). Senescent and young proliferating ECs were subjected to mass spectrometry-based proteomic profiling to identify molecular changes. Additionally, single-cell RNA sequencing (scRNA-seq) analysis was applied to investigate intercellular communication between ECs and human macrophages in 3D co-culture spheroids.
Results: Proteomic analysis revealed upregulation of pro-inflammatory SASP components and enrichment of pathways related to antigen processing and presentation, glycosaminoglycan catabolism, endoplasmic reticulum calcium ion homeostasis, and cellular lipid catabolic processes in senescent ECs. These findings point to profound remodeling of protein turnover, membrane dynamics, and immune signaling in the senescent state. scRNA-seq-based ligand–receptor interaction analysis further indicated altered communication between senescent ECs and macrophages, with increased interaction probabilities for APP–CD74 and GDF15–TGFBR2, alongside decreased interactions involving FN1–CD44 and FN1–(ITGA4+ITGB1), suggesting a shift in adhesion and inflammatory signaling at the immune–vascular interface.
Conclusions: Senescent ECs exhibit proteomic and transcriptomic signatures reflecting immune activation, extracellular matrix remodeling, metabolic stress, and altered calcium signaling. Their changed interaction profile with macrophages suggests a potential role in immune modulation and chronic vascular inflammation.
Acknowledgements: This research was funded by National Science Centre, Poland, under grant No. 2021/43/B/NZ5/03336. Proteomic Analysis Core Facility of the Jagiellonian University is kindly acknowledged for mass spectrometry analysis.

Session 3: Neuroinformatics, computational neuroscience, deep learning: perspectives on computing and the brain

S.03-1. Neuroinformatics, computational neuroscience, deep learning – what's in it for me?

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The development of artificial neural networks since the work of McCulloch and Pitts, through Rosenblatt and others, has followed paralleled the developments of computational modeling of the nervous system since Lapicque, Hodgkin and Huxley, Rall, and others. With time, the role of physical and computational sciences in neuroscience has increased, leading to the proliferation of fields such as theoretical and computational neuroscience or neuroinformatics. Artificial neural networks became part of machine learning, artificial intelligence, and deep learning.
In my talk, I will discuss these concepts and try to organize the relations between them. I will present the different perspectives on neural networks in artificial intelligence and computational neuroscience, the role of deep learning in these fields, and the current and developing fruitful interactions between them. Moderately AI-sceptical and cautiously optimistic, the lecture will set the stage for the following lectures of the session "Neuroinformatics, computational neuroscience, deep learning: perspectives on computing and the brain".
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S.03-2. Can AI open new doors? Ion channels and how to tame them

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In the 70 years since the pioneering work of Hodgkin, Huxley, and Katz, our understanding of the role of ion channels in generating action potentials in neurons has been further elucidated. New ion channels, their subtypes, gating mechanisms, and involvement in regulatory pathways, as well as post-translational modifications, have all been reported. However, the physiological relevance of this rich functional diversity remains poorly understood. In recent work, we identified a critical subset of ion channels that potentially sense metabolic status in neurons and modulate neuronal firing properties accordingly.

This talk will explore why ion channels are crucial for understanding how neurons integrate metabolic signals to regulate electrical activity and physiological function. Drawing inspiration from breakthroughs like AlphaFold, I will present a future roadmap for leveraging AI to predict the interactome landscape of ion channels, thereby understanding their role in cellular function, health, and disease, and ultimately allowing us to 'tame' their often elusive influences for novel therapeutic strategies.

S.03-3. Integrating Computational and AI Strategies to Advance Non-Human Primare Neuroanatomy

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The presentation will illustrate how computational and artificial intelligence (AI) approaches can synergize with traditional neuroanatomy. While neuroanatomy is often perceived as laborious and reliant on expert interpretation, modern computational and AI methods have great potential to facilitate neuroanatomical research by providing streamlined and objective data processing and analysis.
Non-human primate research plays a vital role in bridging the translational gap between rodent models and humans, with the Common marmoset emerging as an increasingly popular model organism. Using the marmoset as an example, I will demonstrate how these approaches can streamline complex neurobiological investigations. I will showcase the computational workflow behind the most comprehensive invasive structural connectivity dataset in non-human primates (http://marmosetbrain.org), which is based on nearly 150 injections into over a half of the cortical areas currently recognized in the marmoset cortex. This foundational dataset has enabled a wide range of interdisciplinary studies, highlighting how computational methods can unlock deeper insights into brain connectivity.
Additionally, I will discuss studies on marmoset cortical anatomy, including the distribution of calbindin-immunoreactive neurons. Lastly, I will present an ongoing project utilizing explainable AI models to predict laminar structures and classify cortical areas within the marmoset brain. Unlike traditional AI applications, this approach employs explainable AI techniques to not only process imaging data but also to interpret the models' decision-making criteria—fostering transparency and deeper understanding.
Collectively, these examples demonstrate that the integration of computational and AI methods with expert neuroanatomy offers a powerful avenue for automating data analysis and enriching scientific insights, leading to more objective and comprehensive understanding of primate brain structure.

S.03-4. What AI can teach us about intelligence

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Background: Understanding the neural basis of mental phenomena remains a great challenge. Recently, great progress has been achieved in artificial intelligence large language models (LLMs) that are already surpassing human abilities in many domains. The notion of intelligence is a complex theoretical construct, and many definitions were formulated. From practical point of view, intelligence is the ability to solve complex problems. What kind of intelligence does AI represent, and in what way do functional processes in the brain resemble those in AI algorithms? Progress in hardware and software inevitably leads to the development of autonomous AI systems capable of setting their own goals, self-reflection, self-improvement, and even emergence of survival instinct.
Materials and Methods: Attractor neural networks provide our best model linking mental states with the physical properties of the brain. Generative neural models based on diffusion processes work in similar way, illustrating associative aspects of thinking in LLMs. Cognitive inspirations provide different reasoning strategies based on search guided by associations. Neural patterns depend on physical properties of networks and neurons, while mental states, often described using psychological constructs, depend on patterns of the whole networks. Attractor network simulations show how temporo-spatial processing disorders can be related to properties of networks and individual neurons, and offer a neural interpretation of various mental disorders.
Results: AI models show many functional similarities to brain processes. The distinction between automatic associative thinking (System 1 of Kahneman) and deliberate reasoning (System 2) is clear. AI models properly primed may learn to mirror human expectations. The development of stable internal models enables imagery, autoreflection, and subjective perception of internal states. Models of continuous thoughts resemble unconscious processes. Cognitive dissonance phenomena in LLMs are similar to those in human experiments.
Conclusions: Despite obvious differences between brains and LLMs, interesting functional similarities of language, personality development, and many aspects of cognitive processes are observed. Benchmarks based on theories of intelligence proposed by cognitive science can be used to test synthetic personalities of conversational agents, including their influence on the ability to solve problems requiring intelligence.

Session 4: The Zebrafish Model: A Tool for Advancing Brain Research

S.04-1. From infection to neurodegeneration - studies on the zebrafish model

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Background: One of the prevailing theories of neurodegenerative diseases identifies neuroinflammation as a leading cause of neuronal damage, implicating microglia as key contributors to the production of pro-inflammatory mediators.
In this study, we used a zebrafish larval model to examine the interaction between the oral pathogen *Porphyromonas gingivalis* (Pg) and microglia, and to analyze Pg ability to induce brain vascular dysfunction.
Materials and Methods: Zebrafish larvae were injected either systemically (into the Duct of Cuvier) or locally (into the hindbrain ventricle) with the wild-type Pg W83 strain, its gingipain-null isogenic mutant ΔK/R-ab, or a vehicle as a control. We assessed larval survival and morbidity, Pg viability and persistence in the brain, interactions with microglia, the kinetics and intensity of neuroinflammation as well as a cerebral vasculature integrity, and behavioral changes.
Results: Systemic infection with wild-type Pg W83, but not with the gingipain-null mutant ΔK/R-ab, resulted in increased bacterial survival in the brain and upregulation of pro-inflammatory genes in both the brain and peripheral tissues. During systemic infection, Pg W83 also caused a reduction in microglial numbers, accompanied by their activation, as indicated by morphological changes and upregulation of activation markers. Additionally, we observed alterations in cerebral vasculature and changes in larval locomotor activity.
In contrast, the ΔK/R-ab mutant was rapidly cleared and did not trigger inflammatory responses, highlighting the pivotal role of gingipains in Pg survival, microglial activation, and neuroinflammation.
Direct hindbrain inoculation of Pg induced only mild, transient inflammation, suggesting that systemic dissemination, and potentially peripheral inflammation, is crucial for affecting the blood-brain barrier and triggering neuroinflammation. Local infection with Pg W83 led to increased microglial numbers and its activation.
Conclusions: We demonstrated that systemic Pg infection can induce peripheral inflammation, neuroinflammation, pro-inflammatory microglial activation, and cerebrovascular damage, all effects largely dependent on bacterial proteases - gingipains.
Moreover, this study highlights the zebrafish larval model as a powerful tool for investigating host immunity–pathogen interactions in the brain.
Acknowledgements: This research was funded by the National Science Centre of Poland (grant no. 2021/43/B/NZ6/00733).

S.04-2. Panta rhei: the use of zebrafish in epilepsy research

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Background: Around 70 million people worldwide suffer from epilepsy, a common and serious neurological condition. With a wide range of etiologies, epilepsy is a spectrum disorder. Despite major advancements in understanding the molecular mechanisms of disease and the release of more than 20 drugs, 30% of patients continue to show resistance to available treatments, which is especially true for rare epilepsy syndromes like e.g. Dravet syndrome.
Although there are many rodent models of seizures and epilepsy, the high cost of maintaining and using these models is a limiting factor. Furthermore, they are not suitable for screening due to, inter alia, the 3Rs principle. The zebrafish has emerged therefore an alternative model in epilepsy research.
Materials and Methods: All experiments were conducted in larval zebrafish up to 7 days post-fertilization. Genes of interest were knocked out (*scn1lab*, Dravet model) or silenced (*cacna1aa*, absence seizures). To induce seizures, chemicals pentylentetrazole (PTZ) or pilocarpine (PILO) were used. Locomotor activity of larvae was analyzed, supplemented with assessment of brain electroencephalographic activity. For deeper phenotyping, histology, RT-qPCR, and confocal microscopy were done.
Results: Our research has shown that deactivation or silencing of gene function (*scn1lab* or *cacna1aa*) results in behavioral changes, which are combined with specific changes in brain imaging. Similarly, administration of pilocarpine results in spontaneous seizures in zebrafish larvae after the wash-out period, which at the behavioral level results in decreased locomotor activity. In screening drug candidates, we have demonstrated the anticonvulsant activity of 6-gingerol, the active ingredient in ginger extract (*Zingiber officinale* rhizome), which has been proven in mice models of seizures.
Conclusions: Zebrafish is a very good model for the study of genetic epilepsy, the study of epileptogenesis processes, and the screening of new anticonvulsant/antiepileptic drug candidates.
Acknowledgements: The studies were financed within Marie Curie-Sklodowska Individual Fellowship (798703-GEMZ-H2020-MSCA-IF-2017), Polish Ministry of Science and Higher Education “Mobilnosc Plus V” program (1649/MOBV/017), Polish-US Fulbright Commission Senior Award 2023/24, NCN grant OPUS 21 (2021/41/NZ4/00337) and Statutory Funds of Medical University of Lublin (DS 448 and 449).

S.04-3. Modeling neuropsychiatric disorders in zebrafish

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Tuberous Sclerosis Complex (TSC) is a rare genetic disease that manifests with early symptoms, including childhood epilepsy and TSC-associated neuropsychiatric disorders (TANDs). The latter comprise anxiety, autism spectrum disorder, and intellectual disability, among others. We showed before that the *tsc2*$^{vu242/vu242}$ zebrafish recapitulate TSC pathology in human patients, as we observed heterotopias and hyperactivation of the mTorC1 pathway in the pallial brain regions, commissural thinning responsible for brain dysconnectivity with delayed axon development and aberrant tract fasciculation, epileptogenesis that resulted in non-motor seizures, and anxiety-like behavior.
However, as TSC patients are often heterozygotes, we also analyzed heterozygotic siblings of the accepted TSC zebrafish model. We discovered that the *tsc2*$^{vu242/+}$ mutants did not suffer from early epileptogenesis and seizures, yet they showed hyperactivation of the mTorC1 pathway in the pallial brain regions and commissural thinning responsible for brain dysconnectivity. Notwithstanding, the heterozygous *tsc2*$^{vu242/+}$ mutants also exhibited increased anxiety-like behaviour, decreased learning and memory, and aberrant development of social behaviour, suggesting that loss of one allele of the *tsc2* gene is enough to cause TANDs-like phenotypes in the zebrafish model of TSC. Our results are in line with the hypothesis that the majority of symptoms in TSC are inherited in an autosomal dominant manner – including TANDs – but for seizure development the loss of heterozygosity is needed.
Acnowledgements: This work was supported by SONATA BIS grant no. 2023/50/E/NZ3/00252 from National Science Centre, Poland.

S.04-4. Role of Tmbim5 in the mitochondrial Ca^{2+} transport in zebrafish brain

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Background: Ca^{2+} in mitochondria can affect cristae shape and control oxidative phosphorylation. In addition, mitochondria serve as cellular Ca^{2+} sinks, making mitochondrial Ca^{2+} uptake important for cellular Ca^{2+} signaling in general and involved in the regulation of important processes such as gene expression and apoptosis. Tissues with high energy demands, such as nervous tissue and muscle, are especially vulnerable to mitochondrial Ca^{2+} misbalance and dysregulation of mitochondrial Ca^{2+} handling has been implicated in various diseases, including myopathies and neurodegenerative disorders. However, the mechanisms controlling mitochondrial Ca^{2+} transport are not fully understood. Tmbim5 has recently emerged as a novel player in mitochondrial Ca^{2+} homeostasis, but its function in Ca^{2+} transport remains to be elucidated.
Materials and Methods: In this study, we investigated the phenotype induced by *tmbim5* knockout in zebrafish. To investigate potential interactions with known Ca^{2+} transport systems – Mitochondrial Calcium Uniporter (mcu) and Na^{+}/Ca^{2+} exchanger NCLX (encoded by *slc8b1*), we generated *tmbim5*/*mcu* and *tmbim5*/*slc8b1* double knockouts. Using this model organism enabled us to combine *in vivo* assays, such as locomotor analysis and Ca^{2+} measurements, with studying Ca^{2+} fluxes under controlled conditions using isolated mitochondria from zebrafish larvae, as well as gene expression analysis.
Results: We observed that loss of Tmbim5 resulted in impaired growth, muscle atrophy, and increased brain cell death. In living larvae, Tmbim5 depletion did not affect mitochondrial Ca^{2+} levels, but reduced mitochondrial membrane potential. *tmbim5*/*mcu* and *tmbim5*/*slc8b1* double knockout lines were both viable without major phenotypes. However, *tmbim5*/*slc8b1* double knockout showed disrupted mitochondrial calcium handling with reduced uptake and efflux. Remarkably, brain phenotypes, such as reduced mitochondrial membrane potential in Tmbim5-deficient brain and elevated cell death were rescued, while muscle dysfunction was exacerbated in the double mutants.
Conclusions: These findings demonstrate that TMBIM5 functions as a calcium efflux pathway cooperating with NCLX in a tissue-specific manner.
Acknowledgements: Funded from National Science Centre, Poland, SONATINA-5 (2021/40/C/NZ4/00031) to IW.

Session 5: Presentation of ERC grants

S.05-1. Presentation of ERC grants

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The European Research Council (ERC) is an organisation of the European Commission aimed at funding excellence in research. The ERC has actively supported cutting-edge and innovative research in all fields of science, including neuroscience.
This session allows researchers to gain valuable insights into the ERC grant application process, providing essential information to maximise their chances of success. Moderated by Dr Janka Mátrai, Scientific Officer at the ERC, the session will begin with an overview of the ERC's funding schemes and key updates for the upcoming application rounds. Next, Professor Sandra Siegert, an ERC Starting Grant and Proof of Concept Grant holder and Principal Investigator of the Neuroscience Lab at the Austrian Institute of Science and Technology (ISTA), will discuss her ERC experience. She will share practical tips and strategies that have helped her succeed.
The session will conclude with an interactive Q&A discussion, allowing participants to engage directly with the speakers to obtain further clarity and knowledge necessary to craft compelling ERC grant applications for advancing their scientific careers.

S.05-2. From the initial discovery of microglia-neuron interplay to founding a start-up

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Background: Microglia, traditionally classified as immune-responsive, invade the brain early and are involved in neural circuit formation and adjustment of synaptic connections. In my talk, I will show how we made an unexpected discovery during the ERC StG.
Results: We found that anesthetic dosage of ketamine in mice initiated a microglia response that enabled the loss of the perineuronal net (PNN), a defined extracellular matrix surrounding parvalbumin-positive interneurons (Venturino *et al.* 2019). This PNN loss shifted adult ocular dominance plasticity and restored juvenile plasticity. Surprisingly, we recapitulated a similar effect with external light entrainment at a selective brain frequency, which reaches the visual cortex through the retina. When we light-stimulated the brain, selective 60-Hz light entrainment replicated ketamine-induced microglia-mediated PNN reduction. Entrainment at 8 Hz or 40 Hz had no effect.
Conclusions: This non-invasive strategy provides an opportunity to explore light entrainment as a therapeutic treatment regime. After initiating intellectual property protection and the successful third attempt at an ERC Proof-of-Concept application, we founded the start-up company, Syntropic Medical, which develops new technologies to exploit our basic research discovery to advance mental health care.

Session 6: New models and tools for stem cell research

S.06-1. Mechanosignaling in human pancreatic beta cell development and function

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Pancreatic beta cells are essential for maintaining glucose homeostasis through insulin production. Their loss or dysfunction is a direct cause of hyperglycemia and diabetes. Consequently, understanding the molecular mechanisms governing beta cell formation and function is crucial for developing new therapeutic strategies.
Leveraging human pluripotent stem cells and multi-omics analysis, our research has uncovered the robust and critical role of mechanosignaling in both the derivation and function of human beta cells. In this presentation, we will detail how various mechanosignaling pathways regulate beta cell fate, replication, and insulin secretion. We will also explore how these pathways operate at different cellular levels and compartments, highlighting their intricate crosstalk with metabolic processes. Our findings underscore mechanosignaling as a fundamental determinant of human beta cell biology.

S.06-2. Brain organoid technologies to model human brain diseases

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Understanding how the human brain function in both health and disease remains one of the greatest challenges in modern science, yet hindered by limited availability of human samples and ethical restrictions.
In recent years, three-dimensional human brain organoids have emerged as a groundbreaking experimental system that overcomes many of these limitations. Derived from human pluripotent stem cells, brain organoids are genetically tractable and capable of recapitulating key aspects of early brain development, including cellular diversity, spatial organization, and functional properties reminiscent of the fetal brain.
In my presentation, I will provide an overview of recent advances in the brain organoid field and showcase several exemplary projects that illustrate how this technology can be leveraged to model human brain diseases. These include applications in the study of neurodevelopmental disorders, neurodegenerative diseases, and brain infections, highlighting the potential of organoids to transform our understanding of brain pathology and support the development of novel therapeutic strategies.

S.06-3. Characterization of the HLA class I landscape in the Lithuanian population for regenerative medicine applications

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Immune matching and rejection pose major hurdles in tissue transplantation. Here, we profile *HLA-A*, *HLA-B*, and *HLA-C* alleles in 3,496 Lithuanian donors genotyped at three-field resolution. The five most frequent alleles constitute 74.6% of *HLA-A*, 43.2% of *HLA-B*, and 59.2% of *HLA-C*, with HLA-A*02:01:01, HLA-B*07:02:01, and HLA-C*07:02:01 being the most common. Lithuanian allele frequencies closely resemble those of populations with pre-Neolithic hunter-gatherer ancestry, such as European-American and British groups. We identified 153 double homozygotes and 51 triple homozygotes for *HLA-A*, *HLA-B*, and *HLA-C*. Compatibility modeling showed triple homozygous profiles match 60.5% of Lithuanians (33.3% for double homozygotes), 13.4% of the British population, and 7.4% of European-Americans. CRISPR-Cas9 guide RNA design yielded 54 candidates predicted to disrupt *HLA-A* or *HLA-B*, while preserving *HLA-C*, producing edited profiles matching over 98.1% of Lithuanians, 95.8% of European-Americans, and 95.6% of the British population. Finally, we established 16 fibroblast lines from double and triple homozygotes, offering a resource for immune-compatibility studies and regenerative medicine applications.

S.06-4. Novel droplet microfluidic methods for single-cell assays

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Background: Water-in-oil droplets generated and manipulated within microfluidic devices offer a powerful experimental platform for ultra-high-throughput assays. In this seminar, I will present our latest microfluidic technologies developed for functional screening and single-cell transcriptomic profiling. The seminar will also highlight high-throughput strategies for functional screening of large single-cell libraries and offer a perspective on how these approaches can be synergistically integrated with AI-driven analytical methods.
Materials and Methods: Our system co-encapsulates barcoded beads and single cells into droplets, followed by fluorescence-activated droplet sorting (FADS) to enrich droplets with viable cells. Cells were sourced from cultures or dissociated mouse embryo brains. Libraries were prepared using the inDrop protocol and sequenced via Illumina NextSeq. Gene expression matrices were generated using zUMIs, with barcode quality control via DropletQC5. Seurat v3 was used to integrate mouse brain datasets.
Results: SpinDrop enhances gene detection rates by up to fivefold and reduces background noise by as much as 50%, depending on sample quality. Leveraging its unique capabilities, we generated a high-resolution molecular atlas of mouse brain development from highly degraded input material. VASA-seq, a novel scRNA-seq protocol, achieves best-in-class capture efficiency of both polyadenylated and non-polyadenylated transcripts across their full lengths. Implemented in a droplet microfluidic format, VASA-seq employs sequential pico-injection steps to perform multistep reactions including cell lysis, RNA fragmentation, poly(A)-tailing, and reverse transcription. Using this approach, we produced the first large-scale total-RNA-seq atlas capturing transcriptomic dynamics during mouse gastrulation and early organogenesis.
Conclusions: SpinDrop and VASA-seq are closely aligned with large-scale atlasing initiatives such as the Human Cell Atlas, as they provide high-resolution, ground-truth reference datasets essential for understanding human biology. Moreover, their combination of low cost, high throughput, and exceptional sensitivity is crucial for accurately capturing the full spectrum of cellular heterogeneity within complex samples.
Acknowledgements: This research was supported by EU Marie Skłodowska-Curie Individual Fellowship (MSCA-IF 750772) and by NCN grant SONATA no. 2021/43/D/ST4/0329.

Session 7: Research methods and modelling in medical applications

S.07-1. Simulations of Life: from Microscale-Organ-on-Chip to Macroscale-Artificial Patient as an Effective Tool for Dynamic Hemocompatibility Assessment

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Background: The heart is formed in the area between the endoderm and mesoderm from the paired valves, right and left, fusing together to form the cardiac coil. The heart covered by the pericardial sac lies asymmetrically in the mediastinum in the pericardial sac. The human heart begins to work within a few days of conception, and from then on it works virtually continuously. It beats 100,000 times a day at a rate that varies according to demand. 40 million times a year; 3 billion times in an average lifetime. It distributes blood and nutrients through some 97,000 km of blood vessels. Unfortunately, this amazing organ can fail, either due to congenital and genetic problems such as inherited heart muscle disease (cardiomyopathy) or secondary to congenital arrhythmias, or due to acquired diseases such as viral infections, coronary artery disease, high blood pressure, drug damage, or secondary to abnormal rhythms.
The project was defined to meet the pressing medical need of effective heart insufficiency treatment of children & teenagers to significantly decrease mortality by miniaturized heart ventricle assist device (VAD) implants. Following the trend to minimally invasive paediatric surgery via vascular access, such placement in an aortic vessel would prevent burdensome invasive surgery at the opened chest for short-term heart support (<4 weeks).
Materials and Methods: A process of modern device fabrication should always be associated with a bio and hemocompatibility investigation of how surface properties modulate its hemocompatibility through plasma proteins adsorption as well as blood cellular elements activation and adhesion. Coatings were be elaborated full filling the following features: (i) ultra-low friction & fully hemocompatible “nano-onion” fullerene/diamond-like carbon PVD hard coatings with (ii) surface functionalization for high adhesion of (iii) athrombogenic oligoproline & polydopamine with superior properties to heparin.
Modelling: Numerical simulations of the testers were performed before testing the materials. As part of the evaluation of the biological suitability of materials dedicated to cardiac support pumps, multi-analyses were performed on several alternative testers and the flow properties, as well as the interactions between the analyzed surface in cells were recalculated theoretically and only in the next step verified experimentally.
Experimental verification: The experimental verification consisted of an originally developed and executed blood-material interaction scenario under microfluidic conditions, using the „Organ-on-chip” technique. The high-speed rotor, self-elaborated and clinically normalized testers “Impact R” and “Symmed” dedicated to evaluation of whole human blood quality changes under dynamic conditions and in the aortic flow simulation assay were proposed in the more advanced step of the material testing. After tests, the aggregates of leukocyte-platelet, platelet-platelet, monocyte-platelet formation, and platelet activation were evaluated. Hemocompatibility was determined in respect to the blood quality analysis using

flow cytometry and the number of activated blood elements attached to the surface by confocal laser scanning microscopy. For the group of selected coatings, the adhesion efficiency of blood morphotic elements was determined under a shear stress by the radial flow chamber assay. This multistep material selection was based on the broad materials characterization and allowed to design artificial blood contacting surface with non-thrombogenic properties. To evaluate the developed materials in the sample scale a new prototype of a bio-pump dedicated for the 3D shape substrate testing was developed. The system enabled the testing of cardiac assist devices under normal and dysfunctional heart conditions. A hydrodynamic evaluation of the prototype was carried out with the use of a hybrid-digital model of the circulatory system.

Results: In most cases, tested coatings showed good or very good haemocompatibility. Type "nano-onion" fullerene/diamond-like carbon PVD hard coatings with coating proved to be superior in terms of activation, risk of aggregation, and the effects of generating microparticles of apoptotic origin, and also demonstrated excellent mechanical properties. The Surface additional surface functionalization for high adhesion of athrombogenic oligoproline & polydopamine resulted in the achievement of activation parameters at the level of the negative control, i.e., disactivated blood.

S.07-2. Generative Models in Biomedical Imaging: From Data Scarcity to Data Abundance

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Background: Generative models have emerged as transformative tools in biomedical imaging, addressing challenges such as limited annotated datasets, modality translation, and image enhancement. Recent advancements, including conditional GANs (cGANs), Variational Autoencoders (VAEs), and diffusion models, have significantly improved image realism and clinical utility. A novel contribution to this field involves using Mixture-of-Experts conditional GANs (MoE-cGANs) tailored for RGB biomedical image synthesis, particularly in hematological diagnostics.
Materials and Methods: This study introduces a MoE-cGAN architecture for generating synthetic blood cell images using the BloodMNIST dataset. Microscopic RGB images were analyzed via k-means clustering and UMAP to assess color-based class separability. A custom cGAN framework with residual blocks and LeakyReLU was developed, incorporating a histogram-based loss function focused on red and green channels. The generator ensemble, guided by a gating network, was trained to reconstruct full RGB images, with Spearman correlation used to optimize channel selection.
Results: The red and green channels were identified as critical for class discrimination, while the blue channel had minimal impact. The proposed MoE-cGAN achieved a classification accuracy of 0.97 on synthetic images using a ResNet50 model. Performance metrics showed an average precision of 0.96, recall of 0.97, and F1-score of 0.96. The Fréchet Inception Distance (FID) score of 52.1 indicated high fidelity to the real data distribution.
Conclusions: The integration of expert-based generation and color-aware loss functions enhances the realism and class fidelity of synthetic biomedical images. This approach contributes to more robust model training in scenarios with limited data, supporting diagnostic accuracy and the scalability of AI in hematology and broader biomedical imaging applications.
Acknowledgements: This work was supported by the subsidy number 16.16.160.557 of the Polish Ministry of Science and Higher Education.

S.07-3. Modelling Transport of Ions using Nernst-Planck and Poisson Equations in Medicine Applications: from Ion Selective Electrodes to Biological Membranes

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The use of ion-selective electrodes (ISEs) in clinical assays is an obvious choice given their inherent characteristics. As they measure in fact the activity, instead of concentration, they are particularly useful in biological and medical applications. ISEs were a response to the growing demand from medical and hospital laboratories for fast, reliable, inexpensive, and fully automated clinical analysis. A breakthrough in clinical applications of electrochemical sensors came in 1966 when Stefanec and Simon pioneered the invention of the valinomycin-based potassium electrode. ISEs are potentiometric ion sensors based on ion-selective membranes and belong to an important group of electrochemical sensors. These sensors are characterized by small size, portability, low-energy consumption, and low cost. Typical ISEs are based on polymeric membranes and contain neutral and/or charged carriers (called ionophores). Currently, a plethora of their varieties are being constructed for the selective determination of a large number of inorganic and organic ions. The approach based on Nernst–Planck and Poisson (NPP) equations is presented here to model the transient and stationary behavior of ion-selective membrane and explain the formation of membrane potential and its relation to measured concentration (or more properly, chemical activity) of primary ion in the presence of interfering ions. Simulations based on the model allow for determining the influence of parameters, such as ion diffusivity, membrane thickness, permittivity, rate constants, and primary to interfering ion concentration ratios on selectivity and detection limit (with its variability over time) of ISEs.
Mimicking biological ion nanochannels showing pH-regulated properties, the synthetic nanochannels functionalized with polyelectrolyte (PE) brushes have been used as tools for active transport control of ions, fluids, and bioparticles. Such nanochannels are of fundamental significance for the design and development of novel nanofluidic devices. A mathematical and computational model for the surface charge properties and ionic current in a pH-regulated nanochannel is presented. The model takes into account multiple ionic species, the effects of the double-layer overlap, electroosmotic flow, Stern layer, and the surface dissociation/association reactions on the nanochannel walls. The model is based on the Poisson−Nernst−Planck equations with special boundary conditions where surface reactions are coupled with fluxes in the bulk of the nanochannel. Moreover, Navier−Stokes equations are used for computing the fluid velocity field, which is added to the convection term in the classical Nernst–Planck flux. Such an approach is necessary in cases where the geometry of the nanochannel does not allow for the parabolic fluid velocity profile. Results provide insight into the pH-regulated ionic current rectification phenomenon in conical nanochannels and allow a better understanding and design of nanopores functionalized with brushes for pH-tunable applications. Although the biological membrane channels have smaller dimensions than those considered in the model and frequently operate in a single or only small number of molecules mode per cycle which does not warrant the use of continuum equations, we still believe such models can be useful in some biological membranes.

S.07-4. Gradient scaffolds for gradient tissue-hydrogel composites for regeneration of osteochondral defects

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Background: Osteochondral tissue engineering faces the fundamental challenge of recreating the seamless gradient transition from soft articular cartilage to rigid subchondral bone. This complex tissue architecture requires scaffolds that can simultaneously support different cell types while providing progressive mechanical properties across a single construct. While various approaches exist for creating gradient scaffolds, multiple issues must be overcome to achieve functional gradient materials, including maintaining structural integrity across property transitions, ensuring biocompatibility throughout the gradient, and achieving appropriate mechanical matching at each zone.
Materials and Methods: In our work, we used a layer-by-layer approach employing whey protein isolate/chitosan (WPI/CS) hydrogels and polyurethane-saccharide (PUSacch) composites, developed through systematic optimization of individual layer compositions. The four-layered gradient design incorporates varying concentrations of hydroxyapatite (HAp) and graphene oxide (GO) to achieve progressive mechanical property transitions from low-modulus cartilage surface (~0.5-2 MPa) to high-modulus subchondral bone (~15-20 GPa). This gradient functionality was obtained through layer-by-layer casting techniques where uncured solutions partially penetrate pores of previously cured layers, creating continuous interfacial transitions rather than discrete boundaries.
Results: The gradient challenge was successfully overcome, as demonstrated by the scaffolds incorporating PUSacch-based bone-mimetic layers that achieve dynamic mechanical stability with storage moduli spanning the required physiological range. Optimal pore architectures (90-105 µm) facilitate cell migration across gradient zones while maintaining structural integrity. Cytotoxicity studies confirmed that the gradient design supports osteoblast proliferation throughout the construct, while HAp incorporation resulted in Ca:P ratios similar to native bone values across the mineralized regions.
Conclusions: This systematic approach demonstrates that gradient functionality in tissue engineering scaffolds can be achieved through careful selection of materials and fabrication techniques, providing a solution for complex tissue regeneration where seamless property transitions are essential.
Acknowledgments: This research was funded by the National Center for Research and Development, Poland (BioMiStem grant No. STRATEGMED3/303570/7/NCBR/2017).

Session 8: Targeting Serotonin 5-HT6 Receptor for Neuropsychiatric and Neurological Treatment Strategies

S.08-1. Blocking the 5-HT$_6$ receptor-mTOR pathway as a disease modifying strategy in schizophrenia

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Background: Schizophrenia is a devastating mental disorder of neurodevelopmental origin affecting ~1% of the population worldwide. It is characterized by a broad pattern of symptoms, including positive, negative, and cognitive symptoms. Cognitive symptoms are the earlier symptoms observed, are predictive of transition to schizophrenia, and are poorly controlled by currently available antipsychotics. Although improving symptomatic treatments remains an important goal, progress in the disease management requires a shift to novel modes of intervention initiated at the early stage of the disease in high-risk subjects. The serotonin 5-HT$_6$ receptor holds special promise to achieve that goal in view of its control of key neurodevelopmental processes, its high densities in brain regions involved in mnemonic functions, and the pro-cognitive effects of receptor antagonists in numerous paradigms of cognitive impairment.
Materials and Methods: To identify signaling mechanisms underlying modulation of cognition by the 5-HT$_6$ receptor, we combined interactomic screens with biochemical, electrophysiological and behavioural studies in two neurodevelopmental model of schizophrenia of different etiologies, mice treated with phencyclidine (PCP) at the neonatal stage and mice carrying the Disc1-L^{100}P mutation, and a mouse model of cannabis abuse during adolescence (mice chronically injected with Δ9-tetrahydrocannabinol (THC) during adolescence), an important risk factor for developing schizophrenia in adulthood.
Results: The 5-HT$_6$ receptor interacts with the mechanistic Target Of Rapamycin (mTOR) Complex 1 and its stimulation underlies the sustained, non-physiological activation of mTOR measured in the prefrontal cortex of PCP-treated mice, Disc1-L^{100}P mice, and mice treated with THC during adolescence. Furthermore, the early blockade of 5-HT$_6$ receptors or mTOR during adolescence definitively prevents the emergence of cognitive symptoms in the three models. This early blockade also prevents some alterations of the intrinsic properties of prefrontal pyramidal neurons.
Conclusions: Blockade of 5-HT$_6$ receptor-operated mTOR signaling during adolescence, a critical phase of prefrontal cortex maturation, might be a novel disease modifying strategy to prevent the emergence of cognitive symptoms and transition to schizophrenia at the adult stage in at-risk subjects.
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S.08-2. New Insights in ciliary serotonin 5-HT$_6$ receptor signaling

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Background: The 5-HT$_6$ receptor (5-HT$_6$R) is a G protein-coupled receptor (GPCR) that plays a pivotal role in neurodevelopmental processes, including neuron migration, differentiation, and the formation of neuronal networks. It is also involved in the pathogenesis of neurological and psychiatric disorders. Notably, 5-HT$_6$R expression is restricted to the central nervous system, with the highest densities found in structures involved in cognitive functions, including the striatum, hippocampus, and prefrontal cortex. During the initial post-natal week, the 5-HT$_6$R transiently resides at the membrane of neurons and astrocytes cell bodies, interacting with partners involved in the formation of neuronal networks. At the embryonic and adult stages, the 5-HT$_6$R is almost exclusively expressed at the membrane of the primary cilium (PC), where it is part of axo-ciliary synapses. Activation of the ciliary 5-HT$_6$R triggers epigenetic modifications and impacts gene expression, although the underlying mechanisms remain unknown.
Materials and Methods: To unravel the mechanisms by which ciliary 5-HT$_6$Rs influence nuclear processes, we used an AP-MS approach on embryonic stem cells (ESCs) expressing a 5-HT6R bearing an HA tag on its N-terminal domain, and a GFP on the C-terminal tail, under the control of a doxycycline-inducible promoter. Cells were differentiated towards cortical neurons. We immunoprecipitated the receptor after 8 days of differentiation and identified its steady-state partners.
Results: We showed, similar to what is observed *in vivo*, that the 5-HT$_6$R is targeted to the primary cilium of differentiated ESCs, allowing us to specifically identify protein partners of the ciliary receptor. We confirmed the interaction of the ciliary receptor with the mTOR pathway, validating our approach. We showed that the 5-HT$_6$R increases the length of the primary cilia and that this effect involves the activation of the mTOR pathway. Interestingly, we also found evidence of an interaction of the receptor with all major ciliary signaling pathways, including the Sonic Hedgehog pathway.
Conclusions: These studies confirmed that the 5-HT$_6$R is linked to signaling pathways that control neurodevelopmental processes, influencing neurogenesis and synaptic plasticity. The newly identified interactions could help us understand how the ciliary 5-HT$_6$R controls gene expression and how this contributes to its role in pathophysiological processes.

S.08-3. Dual antagonism of 5-HT3 and 5-HT6 receptors as a new strategy for targeting schizophrenia

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Dopaminergic neurotransmission plays a central role in many psychiatric disorders. Consequently, many therapeutic strategies rely on direct interaction with dopaminergic circuits, often offering uneven efficacy and poor tolerability. This trade-off is especially problematic in schizophrenia, where dopaminergic D2 receptor blockade remains the cornerstone of treatment. Although particularly effective in reducing positive symptoms, it often severely worsens cognition and negative symptoms.
The atypical antipsychotic clozapine, characterized primarily by weak D2 and potent 5-HT2A receptor antagonism, has demonstrated superior clinical outcomes. Its complex binding profile has encouraged interest in the role of 5-HT3 and 5-HT6 receptors in mediating antipsychotic and pro-cognitive effects. Based on this, compounds with dual 5-HT3 and 5-HT6 antagonistic properties have been proposed as a promising therapeutic strategy.
In this context, we investigated the in vivo effects of FPPQ, a newly developed compound that selectively blocks 5-HT3 and 5-HT6 receptors, acting as a neutral antagonist. We demonstrated that FPPQ exhibits a favorable activity profile in rats, improving positive-like symptoms (phencyclidine-induced hyperlocomotion, sensory gating deficits, conditioned avoidance response) as well as cognitive deficits (novel object recognition). A corresponding antipsychotic-like effect was observed with the combination of selective 5-HT3 and 5-HT6 neutral antagonists (ondansetron + CPPQ), but not with the combination involving a 5-HT6 inverse agonist (ondansetron + SB399885). Neither of the tested 5-HT6 ligands alone produced comparable efficacy.
Conclusions: Our findings support the therapeutic potential of dual 5-HT3/5-HT6 receptor antagonism and encourage further investigation, potentially paving the way for next-generation antipsychotics or effective adjunctive therapies.
Acknowledgements: Funding was provided by National Science Centre, Poland (2021/43/B/NZ7/02855).

S.08-4. Potential therapeutic use of serotonin type 6 receptor ligands in irritable bowel syndrome (IBS): preclinical evidence.

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Background: In the gastrointestinal (GI) tract, the serotonergic system has been implicated in the control of numerous processes, including motility and visceral pain associated with irritable bowel syndrome (IBS). The main symptoms of IBS include abdominal pain, diarrhea, and/or constipation. Numerous factors contribute to the development of this disease, including increased mucosal permeability, visceral hypersensitivity, and/or disturbances in the brain-gut axis. The search for new pharmacological targets for anti-IBS drugs remains an important issue. Recently, a novel strategy in the modulation of GI motility has emerged which is based on blocking differently activated conformational states of type 6 serotonin receptor (5-HT_6). This prompted us to investigate the contribution of both constitutively active and agonist-stimulated serotonin 5-HT_6 receptors to GI motility.
Materials and Methods: We investigated whether the selective serotonin 5-HT_6 receptor ligands, which behave as full inverse agonist (SB-399885), partial inverse agonist (PZ-1444), or neutral antagonist (CPPQ), could reduce intestinal hypermotility and defecation. We used the colonic bead expulsion test and the model of stress-induced hypermotility to evaluate the effect of the tested compounds on the lower GI motility and defecation pattern. Moreover, we used RNAseq and qPCR to analyze the expression of 5-HT_6 receptors in the gut.
Results: We found that both inverse agonists and the neutral antagonist of 5-HT_6 receptors, administered intraperitoneally at the same doses, similarly reduce defecation in physiological and stressful conditions. The effect of PZ-1444 was significantly potentiated by the co-administration of either SB-399885 or CPPQ. CPPQ co-administration did not attenuate the effect of SB-399885, and this combination did not produce significantly stronger inhibition of defecation than SB-399885 administered alone. Combining PZ-1444 and CPPQ produced the most pronounced effect on defecation.
Conclusions: The present findings confirm the contribution of both constitutively active and agonist-stimulated serotonin 5-HT_6 receptors to intestinal motility and diarrhea and highlight serotonin 5-HT_6 receptors as a promising drug target for the treatment of functional GI disorders.
Acknowledgements: Supported by the Medical University of Lodz [503/1-156- 04/503-11-001] to MS and National Science Center, Poland (no. 2021/43/B/NZ7/02855 to PZ and no. UMO-2020/39/D/NZ7/00149 to MS).

Session 9: Materials for medical applications

S.09-1. The potential of lanthanide upconversion nanoparticles in biomedical applications

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Upconversion nanoparticles (UCNPs)—light-responsive materials—have attracted significant interest in biomedical applications due to their unique ability to convert near-infrared (NIR) light into ultraviolet and visible light (NIR to UV-vis). This conversion is particularly attractive in biomedical applications, as NIR light offers deeper tissue penetration, reduced light scattering, and minimal autofluorescence. Compared to conventional fluorescent dyes and quantum dots, lanthanide-based UCNPs (Ln-UCNPs) exhibit superior photostability, longer emission lifetimes, and lower toxicity. Moreover, they operate efficiently under low-power excitation, minimizing the risk of tissue damage. These features make Ln-UCNPs promising candidates for less invasive and more effective strategies in drug delivery, biosensing, high-resolution imaging, and neural stimulation via optogenetics (OG).
Although several synthesis methods—such as thermal decomposition, co-precipitation, and solvothermal techniques—have been employed to tune the morphology and photoluminescent properties of Ln-UCNPs, they often suffer from low conversion efficiency, limited scalability, and poor reproducibility. The microwave-assisted solvothermal technique presents a compelling alternative, offering rapid and uniform heating, particularly when polar solvents are used; however, these solvents quench the emission.
This work investigates the synthesis of $NaYF_4$ nanoparticles doped with lanthanides and co-doped with transition metals using non-polar solvents—barely explored—and acetylacetonate precursors under closed-vessel MR conditions. Reactions carried out at 290 °C for 30 minutes resulted in spherical, homogeneous α- and β-$NaYF_4$ nanoparticles with sizes ranging from 20 to 80 nm, depending on the precursors used. The inclusion of co-dopants such as Li, Gd, Pt, and Zn influenced the structural and morphological properties, as well as the photoluminescent behaviour, particularly the emission intensity and decay times. These findings emphasize the need for a more comprehensive investigation into the impact of microwave irradiation on reaction kinetics, thereby enhancing our understanding of its role in regulating nanoparticle morphology and emission efficiency.
Acknowledgements: IDUB Dz. 4, project number 9882.

S.09-2. Biofunctional solutions for dynamic cell cultures *in vitro*

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Background: Novel tissue engineering solutions, drug screening, and disease pathogenesis platforms assume using patient-derived cells to form functional tissue fragments. For these constructs to behave as they would in a living body, they need numerous exogenous cues, such as signals from the extracellular matrix or the mechanical and electrical cues coming from their surroundings. Under *in vitro* conditions, these can be delivered by introducing tissue-specific scaffolds and/or stimulating chambers. Within this study, a combination of specifically designed collagen-based scaffolds with a novel electrical stimulation device is used to culture iPSCs-derived cardiomyocytes, with an aim to enhance their maturation.
Materials and Methods: Collagen-based scaffolds were fabricated according to the protocol from our priority claim no. EP4553104A1. Briefly, the collagen was dissolved in a mixture of PBS and DMSO, mechanically homogenized and neutralized, poured into polypropylene dishes, and dried to obtain foils. Collagen solutions were added and mixed with different additives: carbon nanotubes, triiodothyronine, dexamethasone, and fatty acids–albumin complex. The foils were then punched out and fit into custom cell wells of an electrical stimulation device, fabricated according to the protocol described in the priority claim no. EP23172756. The uniqueness of the device lies in its ability to deliver the electrical signal through the scaffold, its modular, flexible, and wireless design, and its autoclavability. Cytocompatibility of all the materials was evaluated by direct seeding of cells (HEK 293T and hiPSC-CM). CMs maturation markers were also analyzed.
Results: Electrically conductive, drug-eluting scaffolds were found to be cytocompatible.
Conclusions: The electrical stimulation chamber allows delivery of the as-designed electrical signals directly through the scaffold, affecting the cellular behavior.
Acknowledgments: This study was supported by the National Centre for Research and Development, grant no. LIDER/7/0020/L-11/19/NCBR/2020.

S.09-3. AI-Based Histology Subtypes Illuminate Molecular Pathways of Fibrosis in MASLD

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Background: Assessing fibrosis in metabolic dysfunction-associated steatotic liver disease (MASLD) from histological slides remains challenging due to the spatial complexity of extracellular matrix (ECM) remodelling. Traditional image-based metrics such as collagen proportionate area (CPA) quantify overall collagen content but fail to capture architectural features that reflect fibrosis progression.
Materials and Methods: We developed a deep learning pipeline to subtype collagen patterns from Sirius red-stained histological slides of the Copenhagen Cohort (CoCo, n=202), identifying reproducible collagen clusters (C0–C6). These clusters were evaluated against fibrosis stage, liver tissue transcriptomic profiles, and circulating blood proteomics using linear models and enrichment analyses.
Results: Collagen texture cluster C5 showed stronger associations with fibrosis stage than CPA. While both the clusters and CPA predicted liver-related events comparably, the clusters demonstrated closer correlations with liver stiffness measurements. Their clearest advantage emerged in the molecular data: clusters C4–C6 revealed omics signatures aligned with fibrotic progression, including upregulated transcripts and circulating proteins involved in ECM remodelling, immune activation, and metabolic dysfunction. These clusters uncovered distinct biological processes that were overlooked by traditional CPA-based assessments.
Conclusions: Our data-driven clustering of collagen morphology refines the histological assessment of fibrosis and uncovers multi-omic signatures that CPA overlooks. These interpretable AI-derived features offer translational potential for patient stratification and biomarker discovery in MASLD.

S.09-4. Hybrid carbon composites as materials that can stimulate neural tissue: comparison of pyrolytic carbon and functionalized carbon nanotubes in a simulated environment

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Background: The development of advanced electrode materials is crucial for next-generation neural stimulation systems, particularly in the context of rising neurodegenerative disease incidence. Achieving long-term mechanical durability, chemical stability, and bioelectrochemical functionality remains a major challenge.
Materials and Methods: This study investigates hybrid carbon composites for deep brain stimulation (DBS) applications, comparing carbon fibers embedded in a pyrolytic carbon matrix (CF/PyC) with an enhanced variant modified by oxygen-functionalized multi-walled carbon nanotubes (CF/PyC-CNT-OH). Electrodes were fabricated using chemical vapor deposition (CVD) and subsequently functionalized via airbrush spraying of CNTs. Characterization involved CV, EIS, SEM, XPS, and mechanical fatigue testing under simulated conditions. Biological evaluation was performed using primary mesencephalic cell cultures. Cytotoxicity was assessed by LDH release, while immunostaining for dopaminergic (TH+) and general neuronal (NeuN+) markers enabled analysis after contact with the materials.
Results: Incorporation of CNT-OH significantly improved the electrochemical performance. CNT-coated samples showed higher charge storage capacity, lower impedance, and greater resistance to degradation compared to unmodified electrodes. Mechanical testing confirmed strong adhesion of the nanostructured layer and high tolerance to cyclic stress. Biological tests revealed no cytotoxic effects and, notably, a pro-survival influence on neuronal cells in proximity to CF/PyC/CNT-OH electrodes, especially within 1000–2000 microns. Durability tests, including simulated aging and accelerated degradation, confirmed the structural and functional stability of the electrodes.
Conclusions: Hybrid CF/PyC-CNT-OH composites demonstrate superior electrochemical, mechanical, and biological properties, positioning them as promising candidates for neural interface applications. Their robust performance and biosafety under simulated in vivo conditions support their potential for long-term, effective neural stimulation therapies.
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Session 10: Brain resilience

S.10-1. Neurogenesis and Depression: Exploring the Therapeutic Potential of Cannabinoid CB2 Receptors and Physical Exercise

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Background: Adult hippocampal neurogenesis (AHN) plays a central role in brain plasticity, learning, and emotional regulation. It is strongly influenced by environmental factors such as physical exercise (PE) and chronic stress. Neural stem/progenitor cells (NSPCs), which give rise to new neurons, reside in specialized brain niches regulated by neurotrophic and endocannabinoid signaling. Cannabinoid type 2 receptors (CB2Rs), devoid of psychotropic effects, have emerged as key modulators of NSPC dynamics. Although previous evidence links CB2Rs to neurotrophic factor actions during PE, their role in the neurogenic and behavioral response to PE under chronic stress remains unclear.
Materials and Methods: We employed complementary in vitro and in vivo models. In vitro, postnatal dentate gyrus-derived neurospheres were exposed to a cocktail of exercise-associated neurotrophic factors to simulate PE. CB2R signaling was modulated pharmacologically to assess effects on NSPC proliferation and differentiation. In vivo, a chronic stress mouse model was used to study the impact of CB2R pharmacomodulation combined with PE. Behavioral assessments were conducted, and hippocampal neurogenesis, BDNF expression, and neuroinflammatory markers were analyzed via immunohistochemistry and molecular techniques.
Results: In vitro, the exercise-mimicking cocktail significantly promoted NSPC proliferation and early neuronal and astroglial differentiation. These effects were partially or fully blocked by CB2R antagonism, while late neuronal differentiation remained unaffected. In vivo, chronic stress impaired AHN and induced cognitive and emotional deficits. PE alone had partial benefits, but when combined with CB2R inhibition, it significantly reversed stress-induced behavioral impairments. This combination restored AHN by increasing cell proliferation and neuronal differentiation, reducing neuroinflammation, and elevating hippocampal BDNF levels.
Conclusions: CB2Rs play a crucial role in modulating the neurogenic and behavioral effects of PE, particularly under chronic stress. They are essential in the early stages of neurogenesis and mediate key anti-inflammatory and neurotrophic responses. These findings support the therapeutic potential of combining lifestyle interventions like PE with targeted cannabinoid modulation for treating stress-related disorders.
Acknowledgements: This work was supported by HORIZON-WIDERA-2023-ACCESS-04-01 under grant agreement 101160180 (PANERIS).

S.10-2. Adult neurogenesis and stress resilience

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Post-traumatic stress disorder (PTSD) is a neuropsychiatric condition that can develop after a traumatic experience. Contrary to the common view that PTSD reflects an exaggerated memory of trauma, patients actually exhibit a specific memory profile: heightened emotional recall of sensory details (hypermnesia), alongside impaired contextual memory (amnesia). This imbalance appears central to the disorder, and recent studies suggest that poor contextual encoding may be a core feature of traumatic memory. However, the underlying neurobiological mechanisms remain poorly understood.
Adult-born neurons in the hippocampus—generated through adult hippocampal neurogenesis (AHN)—are known to support contextual memory, pattern separation, and stress resilience, all of which are altered in PTSD. Using optogenetics in a mouse model that distinguishes between normal and pathological fear memories, we showed that activating or inhibiting these neurons could, respectively, prevent or induce PTSD-like memory formation.
Importantly, not everyone exposed to trauma develops PTSD, pointing to risk factors such as early-life stress**,** including prenatal stress. Prenatal stress has been shown to reduce AHN in mice. Building on this, we hypothesized that prenatal stress increases susceptibility to traumatic memory through its impact on AHN. Mice exposed to prenatal stress exhibited behavioral deficits linked to AHN and developed PTSD-like memory profiles in our trauma model. Crucially, optogenetic stimulation of adult-born neurons in these mice prevented the development of pathological fear, promoting instead an adaptive memory.
In conclusion, this research highlights the critical role of adult-born hippocampal neurons in traumatic memory and suggests that disrupted neurogenesis may underlie the vulnerability induced by prenatal stress, offering new preventive and therapeutic avenues for PTSD.

S.10-3. Cravings from Within: Gut Microbiota Drive the Development of Food Addiction

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Food addiction is defined by the loss of control, high motivation, and compulsive intake of highly palatable food. Scientists acknowledge that food addiction shares similar neurobiological mechanisms with drug addiction; however, the exact mechanisms and potential contributors to the development of food addiction are not well understood. Recent evidence points to the gut microbiota as a contributing factor to a range of systemic and CNS disorders. Indeed, a link between the gut microbiota and eating disorders, as well as addictions, has been previously demonstrated; however, the associations with food addiction have been scarce. Therefore, we aimed to investigate the potential link between the gut microbiota and the development of food addiction. First, to determine food addiction in humans, we have used a 35-item Yale Food Addiction Scale (YFAS 2.0) questionnaire, which is adapted from the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5), criteria for substance use disorder. In parallel, we have investigated mice's food-addictive behaviour using operant chocolate-flavoured pellet self-administration sessions. Ultimately, we have investigated the composition of the gut microbiota in both mice and humans, confirming its alterations in association with the diagnosis of food addiction. Specifically, substantial differences were observed at the genus level, with the *Blautia* genus being significantly decreased in both food-addicted humans and mice, demonstrating its potentially preventive role in the development of food addiction. This effect was further explored in a mouse food addiction paradigm, where mice were given *Blautia wexlerae* as a potential probiotic and rhamnose or lactulose, the non-digestible carbohydrates that increase the abundance of the *Blautia* genus, as potential prebiotics. Results demonstrated a substantial reduction in compulsivity-like behaviour and the overall decrease of addictive phenotype of mice in response to *Blautia wexlerae*, rhamnose, and lactulose supplementation. Taken together, our study highlights the crucial involvement of the gut microbiota in food addiction, demonstrating the potential of microbiota-based interventions in preventing and treating food addiction and related disorders.

S.10-4. Shaping Brain Resilience to Stress: The Roles of Sex and Blood–Brain Barrier Dynamics

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Background: Blood–brain barrier (BBB) dysfunction has been increasingly implicated in stress-related disorders, which often differ in prevalence and outcomes between sexes. While stress contributes to the development of these conditions, individuals may also engage adaptive mechanisms that promote resilience. Understanding the interplay between stress, BBB function, and sex-specific responses is essential to uncover the biological basis of susceptibility and resilience.
Materials and Methods: In both males and females, peripheral, BBB, brain, and behavioral responses to stress were evaluated, primarily using ELISA, RT-qPCR, western blotting, and permeability tracers in rodent models of early-life stress (ELS) and chronic unpredictable mild stress (CUMS).
Results: ELS did not exert a clear negative impact on the studied BBB parameters, with observed effects being age-, brain region-, and sex-dependent. Nevertheless, juvenile males appeared more sensitive to ELS. Moreover, ELS modulated BBB and neuroinflammatory responses to immune challenge in a sex-dependent manner. In males subjected to ELS, this response was enhanced, while in females it remained unchanged or even blunted, especially in adulthood. Although CUMS elicited a physiological response in both sexes, females exhibited a more stable and adaptive behavioral phenotype than males. CUMS also altered BBB function in a sex-specific manner.
Conclusions: The results indicate that the regulation of BBB function and inflammatory processes may play a key role in determining susceptibility or resilience to stress, highlighting distinct stress response strategies between sexes and reinforcing the importance of including sex as a biological variable in stress-related research.
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Session 11: Computational medicine and AI: from neuroinspiration to clinical applications

S.11-1. Neuromorphic Computing for Medicine: Opportunities and Challenges

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Neuromorphic computing, inspired by the architecture and functioning of biological neural systems, constitutes a new, and potentially groundbreaking approach to information processing in computer systems. Its potential is specially visible in medical applications, particularly neurology - a discipline that operates on complex, nonlinear, dynamic processes in the brain and neural system. Neuromorphic computing systems rely on the idea of impulse (spike-based) data processing. Spiking Neural Networks (SNN) offer new opportunities for modeling neuronal activity, neurophysiological signal analysis, or multimodal data-based prediction of neurological states.

In contrast to classical digital systems, which use sequential data processing and binary representation, neuromorphic systems are organised around the idea of distributed, parallel, and time-dependent impulse (spike) processing - short electrical signals similar to neuronal action potentials.

Key challenges in neuromorphic computing include: impulse modeling (spike-based computing), creation of artificial neurons and synapses as physical software objects, time-coding of information, synaptic plasticity and time-based learning, energy efficiency, and dedicated hardware (neuromorphic platforms).

The presentation will cover the key principles behind SNNs, address their biological plausibility, and outline future development vectors. Participants will be introduced to mechanisms such as temporal dynamics of the signals, synaptic plasticity, and the influence of neuromodulators - factors that make neuromorphic systems a unique tool for brain modeling.

Emphasis will be placed on the fact that while neuromorphic computing is an intensively developed area of knowledge, its clinical applications are still experimental. Most of its potential functions, from diagnostic support to adaptive rehabilitation systems, are still in the future and require further research, validation, and integration with medical practice.

S.11-2. In silico foundations for AI: Engineering perspective from biomedical device development

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In silico simulation has evolved from a supplementary research tool into a critical component of biomedical science and clinical decision-making. My work has long focused on simulation, often in healthcare-related projects—beginning with modelling thermoregulation in premature infants and later expanding to brain cooling and tissue heat transfer. Early simulations were slow and complex, taking hours per scenario. A breakthrough came with the wider use of reduced-order models: while full simulations remained time-intensive, post-processing allowed simplified models to return results in seconds. This greatly improved usability, even for non-engineers. More recently, artificial intelligence (AI) and machine learning (ML) have further enhanced adaptability, speed, and personalization, aligning with healthcare's digital transformation.

Practical applications and case studies, particularly involving medical devices, illustrate how AI and ML are now integrated into simulation workflows. Their greatest impact lies in reducing simulation time, though improvements in model accuracy and patient-specific customization are also evident. These trends are demonstrated across a variety of examples, from basic 0D models to complex hybrid 1D–4D systems.

A key milestone in this evolution has been improved end-user accessibility. Simulations that once took up to six hours can now be explored in seconds using reduced-order approaches. The introduction of GPU computing allowed thousands of cases to run in parallel, boosting both speed and model robustness. AI and ML have gone further—optimizing solver settings, improving initialization, and accelerating convergence—while also extracting insights from increasingly rich simulation datasets. Collectively, these advances have enabled the development of digital twins: virtual replicas of physiological systems that are now feasible for real-world clinical use.

Simulation has matured from a research-heavy process to a fast, intelligent, and practical tool. While GPU acceleration and ROMs laid the foundation, AI and ML have made simulation broadly accessible by transforming complex workflows into user-friendly applications. This progress now paves the way for digital twins to become central in delivering personalized, predictive, and preventive healthcare.

Acknowledgements: I would like to acknowledge the support and collaboration of colleagues at ANSYS for their contributions to the advancement of in silico simulation.

S.11-3. Predicting Neuropsychiatric Conditions from EEG Signals Using Large-Scale Data

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The utilization of EEG to aid psychiatric and neurological diagnosis is a rapidly expanding field in medical AI. However, many studies in this area face a critical issue: they typically rely on small datasets (often fewer than 100 participants), significantly limiting their potential for generalization to the broader population.
Our approach aimed to address these limitations and develop a more robust algorithm. Using archival data from a psychiatric hospital, we compiled a database of over 13,000 resting-state EEG recordings from patients with a wide range of disorders. Here, we present findings from a one-versus-rest classification of dementia and alcoholism, disorders that exhibited the most prominent EEG disturbances in preliminary analyses. Due to our extensive dataset, we were able to stratify the data by sex, age, and medication use. We extracted a larger number of features from these signals and trained classical machine learning algorithms, achieving a mean accuracy of 75% and 71% (dementia and alcoholism, respectively) in independent patients. Notably, accuracy improved with the model's activation function value, enabling us to evaluate the model's predictions effectively. Critically, we validated the trained algorithm on a novel database of over 10,000 subjects, demonstrating strong generalization capabilities. Our results highlight the importance of large-scale EEG datasets in developing clinically applicable diagnostic tools.

S.11-4. Anti-Virulence Therapy for Bacterial Infections: Can AI Provide the Solution?

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The use of therapies that block bacterial virulence in the treatment of infections is currently a leading direction in microbiological research. The presentation will attempt to identify areas where AI can support this research, as well as those where the capabilities of AI still appear to be very limited.
Over the past two decades, research into anti-virulence therapies has emerged as a leading paradigm in the fight against antibiotic-resistant bacterial infections. The concept (disarming pathogens instead of killing them) offers a promising alternative to traditional antibiotics. While Artificial Intelligence (AI) has already accelerated antibiotic discovery, its potential remains untapped in the field of anti-virulence therapy.
In this talk, I will highlight potential areas where AI could support efforts to understand bacterial virulence and develop targeted anti-virulence strategies. However, I will also discuss the key challenges that hinder the application of AI in this field, and ask if these obstacles can ever be overcome.

Session 12: Novel diagnostics methods in medicine

S.12-1. 6 years of experience in the development of AI in Veterinary medicine. Challenges and opportunities

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Over the past six years, the integration of artificial intelligence (AI) into veterinary medicine has led to significant advancements, particularly in diagnostic procedures. Projects like CyfroVet, developed by the AGH University of Science and Technology's Academic Computer Centre CYFRONET, exemplify these innovations. CyfroVet aims to expedite cytological examinations, which are crucial for detecting neoplastic changes in animals. Traditionally, obtaining results from such tests could take from several days up to two weeks, with high costs. By employing AI algorithms to analyze images of cytological samples, CyfroVet reduces diagnostic time and assists veterinarians in making prompt decisions regarding further diagnostic and therapeutic steps. The system has achieved a classification accuracy of up to 96% for specific neoplastic changes, including mastocytoma, histiocytoma, and lymphoma. This development underscores the potential of AI to enhance diagnostic accuracy and efficiency in veterinary practice.

S.12-2. Pancreatic Segmentation Techniques: Bridging Medical Imaging for Accurate 3D Visualization in Preoperative Planning

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Background: Recent advances in medical imaging quality and resolution have significantly improved 3D organ reconstruction, currently achievable with many user-friendly software tools. However, their limited accuracy often leads to imprecise segmentation, which is especially critical in pancreatic cases where detailed visualization of surrounding vessels is vital for preoperative planning.
Materials and methods: Segmentation of pancreas and surrounding structures was performed using the open-source software 3D Slicer (version 5.7.0, release date: 2024-10-28). Both automatic and semi-automatic methods were evaluated: the automatic method applied the Whole Body option on CT series, while the semi-automatic method identified regions by Hounsfield Units, with manual corrections based on image review and anatomy expertise. Segmentation reliability was confirmed through independent radiologists review. The goal in both approaches was to capture spatial relationships emphasized by the surgeon, such as vessel positions relative to nearby organs and pathology, closely running arteries prone to being overlooked, absence of aneurysms, infiltration limiting reconstruction, and vessel topology. Imaging quality affected by low contrast and patient anatomical variability significantly impacted segmentation. Therefore, it was stated as the other assessment of the visualization adequacy for preoperative planning.
Results: The evaluation showed that fully manual segmentation is too time-consuming, while the automatic method has significant restrictions, including not visually appealing results, but what is more important – trained on a healthy database – it may not include critical vascular details, and it is often limited to major arteries. In contrast, the semi-automatic approach optimizes segmentation time and allows consideration of surgical aspects like vessel-pathology relationships, enhancing confidence in identifying potential intraoperative challenges.
Conclusions: The use of 3D reconstruction techniques of anatomical structures helps bridge the gap between raw imaging data and clinically relevant 3D models. Our findings facilitate the integration of segmentation tools into preoperative planning, supporting the diagnosis, enhancing the understanding of the spatial anatomical relationships and reducing planning time. The proposed method allows quick visualization of key structures, providing operators with essential insights before surgery.

S.12-3. Exhaled breath analysis as a novel diagnostic tool for daily clinical practice

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Early disease detection correlates with improved prognosis and, in many cases, reduced mortality rates, such as in cases of cancers and cardiovascular disorders. The currently used screening techniques are based on invasive biopsies, less invasive blood biomarker analysis, and non-invasive imaging (which are sometimes invasive with contrast). However, all the above-mentioned approaches require well-qualified staff, laboratories, and other necessities. Thus, a non-invasive disease screening technology is the need of the hour, and exhaled breath analysis is a solution that could fulfil this need in many fields. Disease screening by exhaled breath analysis, such as the analysis of exhaled volatile organic compounds (VOCs), can revolutionize healthcare in both the short-term and long-term management of medical treatments. This lecture will provide an introduction to the exhaled breath analysis methodology, including definitions of the alveolar gradient and biomarkers. The potential diseases diagnosed by the most frequently detected biomarkers in breath will also be presented, along with the recently obtained results by the companies.

Session 13: Neuroimaging Innovations: Integrating Bioinformatics for Improved Analysis

S.13-1. Optimizing Neuroimaging Pipelines for Enhanced Translational Insights

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Advances in neuroimaging have significantly contributed to our understanding of brain function and dysfunction. However, the translational impact of many preclinical studies remains limited, often due to inconsistencies in data acquisition, processing, and analysis methodologies. In this talk, I will discuss how systematic optimization of the entire neuroimaging pipeline—from experimental design and data acquisition to processing and statistical analysis—can substantially improve reproducibility and enhance the translational relevance of preclinical research.

Drawing from practical experience developing cloud-based platforms for both clinical and preclinical neuroimaging, I will highlight common sources of variability and introduce scalable solutions that promote standardization and quality control. Emphasis will be placed on integrating robust preprocessing workflows, automated quality metrics, and harmonized analytical approaches that facilitate meaningful comparisons across species and studies.

By streamlining neuroimaging workflows and ensuring methodological rigor, we can better bridge the gap between experimental models and human applications, ultimately accelerating the development of effective diagnostics and therapeutics for brain disorders.

S.13-2. Current Analysis Methods in Positron Emission Tomography: An Overview

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Positron emission tomography (PET) imaging has become indispensable in biomedical research, offering unique insights into molecular processes, disease mechanisms, and therapeutic interventions. Traditional quantitative analysis methods in PET include kinetic modelling and standardized uptake value (SUV). While these approaches have served as the foundation for PET studies, they often capture only a limited fraction of the information available in the images and may miss subtle changes. Recently, the field has experienced a paradigm shift by integrating advanced computational methods, particularly radiomics and artificial intelligence (AI). On the one hand, radiomics - the extraction of quantitative features from medical images- enables the characterisation of tissue heterogeneity and morphology that are not readily apparent to the human eye. These features can capture complex spatial distributions of signal intensities and pixel interrelationships, providing enhanced biomarkers for disease characterisation and treatment response prediction.
On the other hand, deep learning algorithms excel at automated feature extraction and pattern recognition, offering superior accuracy in lesion detection, tumour segmentation, and disease classification. Unlike traditional approaches that sometimes require manual feature selection, deep learning models automate this process through neural networks with multiple layers, enabling more sophisticated analysis of complex radiological data. These current applications of AI in PET extend beyond image interpretation to include noise reduction, image reconstruction optimisation, and dose reduction strategies.
Despite these methods can enhance image quality while reducing radiation exposure (making studies more efficient and ethically sound), several challenges persist in implementing these in the current practice: the scarcity of annotated datasets, sensitivity to acquisition parameter variations, and issues with model explainability and reproducibility remain significant limitations. Additionally, integrating multimodal data (e.g., PET-MRI) and standardisation across different imaging platforms presents ongoing technical challenges. This presentation, therefore, will provide a comprehensive overview of current analysis methods employed in PET, highlighting both traditional approaches and new perspectives.

S.13-3. Imaging transcriptomics of genetically altered mouse models of brain disorders

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Background: Magnetic resonance imaging is a powerful, versatile tool used for everything from clinical diagnosis to characterization of preclinical disease models. However, one limitation of MRI is its low biological specificity. Imaging transcriptomics is an emerging field in which neuroimaging data is integrated with brain-wide gene expression atlases to gain insights into the molecular basis of imaging-derived phenotypes.
Imaging transcriptomics was applied to an ex vivo MRI dataset acquired from a cohort of the PLP-αSyn mouse model of multiple system atrophy (MSA). In these transgenic mice, wild-type (WT) human alpha-synuclein (αSyn) is expressed in oligodendrocytes under the proteolipid protein (PLP) promoter.
Materials and Methods: The heads of nine female PLP-αSyn mice and nine age-matched WT females were imaged ex vivo in a 9.4T MRI scanner using a protocol that included T2-weighted imaging and diffusion tensor imaging (DTI). Tensor-based morphometry was performed to look for voxel-wise volumetric differences between PLP-αSyn and WT brains. Voxel-wise analysis was also performed on DTI-derived fractional anisotropy (FA) and mean diffusivity (MD) maps. The t-statistic maps resulting from the voxel-wise tests on these MRI parameters (volume, FA, and MD) were taken as the imaging-derived phenotypes for spatial transcriptomic analysis using the coronal gene expression maps of the Allen Mouse Brain Atlas. Gene set enrichment analyses were performed using cell-specific expression gene sets and the Gene Ontology (GO) biological process annotations.
Results: Ex vivo MRI revealed widespread atrophy in PLP-αSyn brains in regions involved in human MSA, including the cerebellum, pontine tegmental nuclei, and the substantia nigra. FA was reduced in and around various white matter structures, while MD was increased in various parts of the brainstem and cerebellum.
Imaging transcriptomic analysis showed that genes specifically expressed by oligodendrocytes and genes involved in oligodendrocyte development and myelination were overrepresented among the genes with expression patterns correlated strongly with volume and FA reduction. No cell type or GO term was enriched for genes with expression patterns correlated with MD changes.
Conclusions: The ex vivo MRI phenotype is consistent with previous studies on the PLP-αSyn mouse and the neuropathology of MSA. This includes nigral degeneration, olivopontocerebellar atrophy, and potential demyelination indicated by decreased white matter FA. The strong spatial correlation between the MRI phenotype and expression patterns of genes related to oligodendrocytes and myelination suggests that these brain changes are closely linked to the oligodendrocyte-specific overexpression of αSyn.

S.13-4. Promoting Reproducibility in Biomedical Research: Navigating the Landscape of AI and BioImage Informatics

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Reproducibility is a cornerstone of robust biomedical research, yet challenges persist, particularly in the complex domains of AI and bioimage informatics/analysis. In this presentation, I discuss the critical need for enhanced reproducibility in image-based biological studies and detail some strategies implemented at the Francis Crick Institute. Our Image Analysis Group aims to empower researchers with the tools and knowledge to independently analyse their data, focusing on key pillars: training, standardization, informed interpretation, and self-sufficiency. Through dedicated workshops, we have taught hundreds of researchers fundamental image analysis concepts, emphasizing the impact of image quality and appropriate quantification on data reliability. I will also showcase some practical examples of our work and the challenges associated with ensuring they can be used reproducibly by others. Finally, I will discuss our latest work on common pitfalls in image data interpretation, highlighting the importance of robust statistical methods and adequate sample sizes to accurately describe populations and discern subtle biological differences.

Session 14: Personalized medicine-innovative treatments for civilization diseases

S.14-1. OGF and Poplar-Derived Compounds: A Dual Approach to Reprogram PDAC

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Background: Pancreatic ductal adenocarcinoma (PDAC) ranks among the deadliest cancers, largely due to its late diagnosis, aggressive progression, and marked resistance to standard chemotherapy. This resistance arises from PDAC's genetic heterogeneity, complex tumour biology, and an immunosuppressive microenvironment, emphasizing the urgent need for novel therapeutic strategies. Opioid growth factor (OGF, [Met^5]-enkephalin) has demonstrated antiproliferative effects in pancreatic cancer models, particularly when combined with chemotherapeutic agents. Concurrently, flavonoids derived from poplar propolis—such as pinocembrin, pinostrobin, tectochrysin, and galangin—have shown promising anticancer activities, including antioxidant, pro-apoptotic, and anti-metastatic effects. This study aimed to evaluate the anticancer potential of selected poplar-derived flavonoids in combination with OGF in PDAC.
Materials and Methods: Three human pancreatic cancer cell lines (Panc-1, MIA PaCa-2, and AsPC-1) were treated with individual flavonoids and OGF, both as monotherapies and in combination. Tumour-associated characteristics were assessed using MTT assay, BrdU incorporation, sphere and colony formation, migration, and invasion assays. Apoptosis induction and cell cycle distribution were determined by flow cytometry. Changes in the expression of key proteins and genes were assessed by Western blotting and RT-qPCR.
Results: All tested flavonoids exhibited selective cytotoxic effects on PDAC cells, with enhanced efficacy observed when combined with OGF. Co-treatment significantly inhibited cell proliferation, stemness, and invasiveness. Flow cytometry revealed increased apoptosis and cell cycle disruption following combined treatment. These effects were corroborated at the molecular level by changes in mRNA and protein expression. Among the combinations, pinocembrin with OGF showed the most pronounced anticancer activity across all parameters tested.
Conclusions: The combination of poplar propolis flavonoids with OGF demonstrates synergistic anticancer effects in PDAC models, suggesting a promising, low-toxicity therapeutic strategy. These findings warrant further preclinical studies to explore the translational potential of this dual approach for pancreatic cancer treatment.
Acknowledgements: This study was supported by NCN (OPUS) No. 2019/35/B/NZ7/04212.

S.14-2. The Power of Molecular Targeted Imaging and its Implications for Disease Diagnosis and Personalized Treatment

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Advances in molecular imaging have transformed the way we visualize, quantify, and understand disease processes at the cellular and molecular levels. Unlike traditional anatomical and physiological imaging techniques, molecular targeted imaging enables noninvasive assessment of specific biological pathways and tissue microenvironments, offering profound implications for both the diagnosis and treatment of a wide range of pathophysiological conditions, including cardiovascular disease and cancer.

By integrating advanced imaging modalities such as X-ray computed tomography (CT) with molecular techniques like single photon emission computed tomography (SPECT) and positron emission tomography (PET), we can visualize dynamic molecular processes in vivo. These approaches offer new opportunities for early disease detection, improved risk stratification, and individualized therapeutic monitoring. In cardiovascular medicine, molecular imaging enables the evaluation of processes such as inflammation, thrombosis, myocardial viability, and both naturally occurring and therapeutically induced angiogenesis. In oncology, it facilitates the detection of tumor-specific biomarkers, assessment of heterogeneity within tumors, and prediction of treatment response—all of which are crucial for guiding targeted therapies.

Importantly, molecular imaging plays a critical role in the era of personalized medicine. By enabling patient-specific characterization of disease biology, it supports tailored treatment strategies, more accurate prognoses, and real-time monitoring of therapeutic efficacy. Such individualized approaches not only enhance clinical outcomes but also reduce unnecessary interventions and associated healthcare costs.

This presentation will provide an overview of current and emerging molecular imaging techniques, highlighting their applications in the assessment of cardiovascular and oncologic conditions, and discussing how these technologies support more precise, efficient, and personalized healthcare delivery.

S.14-3. Personalized pharmacotherapy of schizophrenia in relation to observed symptoms

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Schizophrenia is a mental disorder that affects approximately 1–2% of the population and develops in early adulthood. The disease is characterized by positive, negative, and cognitive symptoms. The symptoms can be expressed at different intensities, may vary between individuals, and may change as the disease progresses. A large percentage of patients with schizophrenia have a treatment-resistant disease, and the risk of developing adverse effects is high. Many researchers have attempted to introduce new antipsychotic drugs to the clinic. The diversity of schizophrenic symptoms is one of the main cause of disappointing results in this field. Our recent research indicates that the simultaneous activation of two receptors with sub-effective doses of their ligands induces similar effects as the highest dose of each compound alone. The combinations of the ligands may be adjusted to the symptoms expressed. Some combinations reverse all schizophrenia-related deficits in animal models, but others are active only in select models of schizophrenia symptoms (i.e., cognitive or negative symptoms). Here the potency of the positive allosteric modulators of metabotropic glutamate receptors (mGlu), muscarinic and GABAB receptors will be discussed, with the special attention on their combinations, to reverse schizophrenia symptoms, will be discussed.

Poster Session 1

Clinical research

P.1.1. Optimised computational predictors for pharmacogene variant function and adverse drug reactions risk

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Background: Variation in pharmacogenes such as CYP2C19 - a key enzyme in the metabolism of antidepressants and other drugs - is traditionally captured by star-allele nomenclature, which becomes limited as novel and rare variants accumulate. Existing variant effect predictors struggle to address pharmacogene-specific contexts and to aggregate effects across haplotypes. We introduce PharmGScore and PharmMLScore tools to provide continuous, gene-specific functional scores, which can facilitate precise prediction of drug response and adverse events, especially as next-generation sequencing becomes standard in clinical practice.
Materials and Methods: We comprehensively examined the ability of multiple existing variant effect predictors (CADD, FATHMM-XF, PROVEAN, MutationAssessor, SIFT, Phylop100, APF, and APF2) and our two novel ensemble models to assess the functional consequences of pharmacogenetic variants. First, we evaluated each tool's ability to correctly classify known star alleles (n=541) from the PharmVar database using receiver-operating characteristic curves and calculating the area under the curve (AUC). Further, we extended predictions to all possible amino acid substitutions of *CYP2C19* from a deep-mutational scanning dataset (n=1,381) measuring CYP2C19 protein abundance. Finally, we applied the tools to whole-exome sequences of 200,000 UK Biobank participants and estimated odds ratios (ORs) for adverse drug reactions (ADRs) associated with reduced CYP2C19 function.
Results: For alleles of known phenotype, PharmGScore achieved AUC of 0.85, distinguishing decreased/no-function from normal/increased-function haplotypes, and AUC of 0.95 in CYP2C19 in vitro abundance data, the highest among all evaluated tools. In UK Biobank participants, higher PharmGScore values in CYP2C19 - indicating its reduced functionality - were associated with elevated antidepressant ADR risk (OR 1.24, 95% CI 1.05–1.47), surpassing star allele-based OR 1.20 (95% CI 1.01–1.42).
Conclusions: Ensemble computational predictors, particularly PharmGScore, accurately quantify pharmacogene haplotype function across both experimental assays and clinical sequencing data. Continuous scoring enables the incorporation of rare and novel variants and may enhance pharmacogenomic guidelines and personalised therapy selection.
Acknowledgements: The work was supported by the National Science Centre, Poland, under research project no PRELUDIUM BIS-3 nr 2021/43/O/NZ7/01187.

Preclinical research

P.1.2. Advancing EV Characterization Using U-Net Segmentation: Morphological Insights for Biomarker and Drug Delivery Applications

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Background: Extracellular vesicles (EVs), including exosomes, are nano- and micro-sized double-membrane vesicles secreted by most cell types and found in biological fluids. They are key mediators of intercellular communication, transferring genetic and proteomic material between cells. EVs have emerged as valuable biomarkers in metastatic malignancies and tumors, as their cargo reflects the physiological state of donor cells. They also offer strong potential as drug delivery systems (DDS) due to their biocompatibility, natural targeting ability, and modifiable surfaces. However, characterizing EVs remains challenging due to their heterogeneous size, shape, and distribution.
Materials and Methods: This study presents a machine learning-based U-Net segmentation model to identify and quantify EV morphological features such as size, shape, area, and aggregation. The model is trained and validated using a curated dataset of transmission electron microscopy (TEM) and cryo-electron microscopy (cryo-EM) images of EVs. Preprocessing includes contrast enhancement and noise reduction. The U-Net architecture performs semantic segmentation to delineate individual vesicles and extract morphometric data.
Results: Initial results show that the model accurately segments EVs, even in dense clusters and low-contrast areas. It captures subtle morphological differences essential for vesicle classification. Quantitative comparisons indicate significant improvements over manual annotation in accuracy and processing time, supporting reproducible and scalable EV morphology analysis.
Conclusions: This U-Net-based framework enables robust, automated EV morphological analysis. Future improvements will focus on enhancing generalizability across datasets and imaging platforms. The approach supports computational EV research and advances biomarker identification and targeted DDS development in oncology and beyond.
Acknowledgements: This work was supported by the National Science Centre of Poland (2022/45/B/NZ7/01430, E.Ł.S.) and the Jagiellonian University via CRP/0641.221.2020, SciMat, and qLife (Excellence Initiative – Research University). Additional support came from a stipend from Jagiellonian University Collegium Medicum.

P.1.3. Improving Stroke Triage with EEG-Based Explainable ML in the Prehospital Setting

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Background: Anterior circulation large vessel occlusion (LVO-a) stroke is a time-critical emergency requiring rapid prehospital identification for effective endovascular thrombectomy. EEG holds promise for LVO-a detection in prehospital settings, but EEG-based triage requires fully automated analysis. This study develops an interpretable machine learning framework for LVO-a detection using EEG.
Materials and Methods: We analyzed data from two prospective multicenter studies (ELECTRA-STROKE, AI-STROKE), including 266 ischemic stroke patients (67 LVO-a, 199 non-LVO-a) confirmed via CT angiography. Resting-state EEG (3 min) was recorded using an 8-channel dry-electrode system. Signals were denoised, and a comprehensive set of features was extracted, then selected using Minimum Redundancy Maximum Relevance. Class imbalance was addressed with the Synthetic Minority Over-sampling Technique. Models were optimized via Optuna with 5-fold stratified cross-validation. AUROC was the primary metric (train/test split). An optimal threshold was selected from the ROC curve, prioritizing specificity ≥80% while maximizing sensitivity. Performance was reported using accuracy, precision, recall, and F1-score. Bootstrap validation assessed AUROC stability. SHAP analysis identified the most predictive features.
Results: The LightGBM model emerged as the top performer, achieving an AUROC of 0.91. At the optimized threshold, the model reached an accuracy of 88.9%, with a sensitivity of 92.9% and a specificity of 87.5% for LVO-a detection. Macro-averaged F1-score was 0.87. Bootstrap validation confirmed the model's stability, yielding a mean AUROC of 0.918 (95% CI: [0.867–0.966]). SHAP analysis revealed greater signal variance (Hjorth Activity), increased inter-hemispheric asymmetry (BSI in the alpha band), reduced functional connectivity (WPLI) in the alpha band and increased connectivity in the theta band, lower alpha relative power, and a decreased delta/alpha power ratio as the stronger predictors of LVO-a.
Conclusions: We developed and validated a novel, fully automated dry-EEG pipeline for prehospital LVO-a detection, integrating robust signal processing, feature selection, and interpretable ML. Our framework demonstrated promising results, supporting its potential for real-time clinical application.
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P.1.4. The influence of obesity-inducing diet on rewarding and motivational effects in Wistar rats

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Background: Obesity inducing diet (OID) can be responsible for the disruption of the brain reward system, resulting in weakened response to reward. This study investigated the effects of sex and diet on reward-related behaviors in rodents. The research included sacharose preference test and food self-administration (SA).
Materials and Methods: We used both male and female obesity-prone (OP) and obesity-resistant (OR) rats (n = 9-10 per group) that had been fed an OID for 12 weeks. First, these animals were tested in the sucrose preference test (SPT), followed by assessments of food SA (sweetened milk as reinforcements), motivation for food seeking using instrumental progressive ratio procedures, and reinstatement evoked by food and associated cue (light + tone).
Results: In the SPT, no significant differences between OID rats in the Sucrose Preference Index were found, suggesting that the OID exposure did not induce anhedonia. However, the OID rats showed alternation in food and water intake at the baseline and between SPT conditions. Indeed, OP and OR males increased whereas OP females decreased food intake during baseline. Furthermore, only OP male rats showed decreased water intake during baseline. Interestingly, only OR males consumed more sucrose than OP males. OID rats of both sex showed neither differences in reinforcement nor motivation to receive rewards in SA procedure. During food SA, under fixed ratio (FR1 and FR5) reinforcement schedules, differences were observed only in the number of lever presses, but not in the number of rewards obtained, in both sexes. In the progressive ratio test, diet did not affect motivation, as indicated by an unchanged breaking point across all groups. During the reinstatement phase, OP females showed a greater cue-induced reinstatement response compared to the OR group. In contrast, OP males displayed lower vulnerability to food-induced reinstatement relative to the SD group.
Conclusions: These findings highlight that the long-term OID feeding did not evoke anhedonia but increased vulnerability to sucrose intake specifically in OR male rats suggesting reward system sensitivity. Furthermore, OID exposed rats showed alterations in food seeking-behavior. Overall, these results support that exposure to OID diet may change sensitivity to natural rewards depend on obesity susceptibilites.
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P.1.5. Effects of psilocybin in the rat model of chronic unpredictable mild stress

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Background: Psilocybin belongs to the natural origin tryptamine class of psychedelics. It is already recognized as a putative antidepressant and anxiolytic drug that has been shown in a number of clinical trials. However, its pharmacology and neurobiological mechanisms are not well understood. To address this issue, we have investigated the effect of psilocybin on rats subjected to chronic unpredictable mild stress (CUMS).
Materials and Methods: In CUMS model, animals were subjected to a variety of mild stressors (food and water deprivation, cage tilting, grouped housing, soiled cage, stroboscopic illumination, intermittent illumination) applied randomly for several weeks. Sucrose tests as a measure of anhedonia were performed weekly until the consumption of sucrose solution by the stressed animals fell below ca. 40%. Psilocybin was administered twice at the dose of 0.6 mg/kg at a weekly interval. Anxiolytic effect was examined using light darkbox (LDB) and elevated plus maze (EPM) tests. Antidepressant effect was assessed using the forced swim test (FST) while the motor activity of the animals was examined in the open field (OF) test. Neurogenesis in the hippocampus was studied using immunoenzymatic methods. All tests were performed one week after the last dose of drug administration.
Results: Psilocybin reversed anhedonia in rats, showed anxiolytic effect in LDB and EPM tests. It also affected animal's activity in the OFtest and produced an antidepressant effect in the FST. Furthermore, psilocybin increased proliferation and neuron development in the rat's hippocampus.
Conclusions: The data show that psilocybin, as agonist of serotonin 5-HT2A and 5-HT1A receptors, is able to induce neuroplastic changes in the brain resulting in reversal of depressive-like symptoms in CUMS animal model of depression.
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P.1.6. The effect of ketamine on mGluR5 receptors in a model of treatment-resistant depression: an autoradiographic study

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Background: Metabotropic glutamate receptor 5 (mGluR5) plays a key role in regulating synaptic plasticity, stress responses, and the pathophysiology of depression. There is growing evidence linking mGluR5 to the mechanism of action of ketamine, a rapid-acting antidepressant (RAAD), although its molecular effects remain only partially understood.
This study aimed to assess the impact of repeated ketamine administration on mGluR5 receptor binding in selected brain regions in the chronic mild stress (CMS) model of treatment-resistant depression (TRD) using Wistar-Kyoto (WKY) rats.
Materials and Methods: Adult male WKY rats were exposed to a 10-week CMS protocol. During the last 7 weeks, animals received injections of either ketamine (10 mg/kg, i.p.) or saline once per week. After treatment, autoradiographic binding of mGluR5 receptors was assessed in coronal brain sections using [^{3}H]-MPEP. Brain regions analyzed included the prefrontal cortex (PFC), ventral and dorsal hippocampus, and the septum.
Results: Behavioral assessment using the sucrose preference test revealed a robust stress-induced anhedonia in WKY rats subjected to CMS, evidenced by a sustained reduction in sucrose intake. Ketamine administration significantly reversed this effect in the stressed group, restoring sucrose intake to levels comparable to non-stressed controls. No changes were observed in sucrose consumption in non-stressed animals receiving ketamine, indicating a stress-dependent, selective antidepressant-like effect. In ketamine-treated CMS animals, we observed a significant reduction in mGluR5 receptor binding in all examined brain regions compared to stressed saline-treated controls. The most pronounced decreases were found in the PFC and hippocampus. No significant changes in receptor binding were observed in non-stressed animals, suggesting a stress-dependent modulatory effect of ketamine on mGluR5.
Conclusions: Our findings suggest that ketamine downregulates the availability or expression of mGluR5 receptors in limbic regions critically involved in mood regulation, particularly under chronic stress conditions. This may represent a key component of ketamine's antidepressant mechanism by attenuating excessive glutamatergic signaling and facilitating synaptic plasticity. These results support mGluR5 modulation as a potential therapeutic target in TRD.
Acknowledgements: The study was funded by Statutory Activity of the Department of Pharmacology, Maj Institute of Pharmacology PAS.

P.1.7. Heritability of maternal high-fat diet-induced microbiome changes and strategies for its reversal

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Background: Maternal obesity is increasingly linked to neurodevelopmental disorders in offspring, with emerging evidence showing that gut microbiota dysbiosis (GMD) - transmitted from mother to child - may disrupt brain development and increase risks for conditions like autism spectrum disorder (ASD). In this study, we investigated whether a maternal high-fat diet (HFD) induces GMD and whether these microbial alterations are inherited by the offspring. Furthermore, we explored the potential of two interventions during pregnancy and lactation — using either a probiotic or an anti-diabetic agent — to prevent the transmission of dysbiosis associated with maternal obesity to the offspring.
Materials and Methods: Female C57BL/6J mice were fed an HFD (45% energy from fat) for 8 weeks before mating and continued on this diet until weaning of the offspring. During pregnancy and lactation, dams received one of the following treatments: Lacidofil (6×10^9 CFU/day) or metformin (50 mg/kg/day). The control group was fed a control diet (10% energy from fat) without any medical treatment. DNA extracted from fecal samples (mothers and offspring at PND 28; n=12/ group) underwent shotgun metagenomic sequencing, with microbial taxa profiled and diversity and taxonomic markers and associations analyzed.
Results: The maternal microbiome was mainly shaped by diet, with an added influence from the interaction of diet and treatment. In contrast, the offspring's microbiome was primarily influenced by the combined interaction of maternal diet and treatment, rather than by diet alone. Alpha diversity remained stable in mothers but decreased significantly in offspring after maternal HFD, showing sex-specific differences. Only a limited number of maternal diet-related taxonomic markers appeared in offspring.
Conclusions: In offspring, a maternal HFD reduces microbial diversity and/or evenness, suggesting less complex and potentially less resilient gut communities. Differences in partial alpha diversity restoration in female and male offspring suggest a sex-specific microbiome response to the interventions during pregnancy and lactation.
Microbial signatures did not transfer consistently from mother to offspring; however, trends in taxa abundance related to dietary treatment were still observed.
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P.1.8. Context-dependent miRNA regulation in treatment-resistant depression after ketamine treatment

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Background: MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally and have been implicated in the pathophysiology of depression and antidepressant response. Their stability in peripheral fluids makes them attractive candidates for biomarkers of drug efficacy. Ketamine, a rapid-acting antidepressant (RAAD), has shown promise in treatment-resistant depression (TRD), but its underlying molecular mechanisms remain incompletely understood. In this study, we analyzed serum miRNA profiles in a rat model of TRD to identify miRNA signatures associated with repeated ketamine administration.
Materials and Methods: Adult male Wistar-Kyoto (WKY) rats, known for their genetic vulnerability to depressive-like behavior, were divided into two experimental paradigms:
(1) control WKY rats receiving saline or ketamine (10 mg/kg, i.p.) once weekly for 7 weeks;
(2) WKY rats were subjected to a 10-week chronic mild stress (CMS) procedure, with ketamine or saline administered during the final 7 weeks. Anhedonia was assessed via sucrose preference testing. Serum samples were collected 24 h after the final treatment, and expression of 32 pre-selected miRNAs (based on literature and pilot screening) was analyzed using TaqMan Array Cards and qBase+ software.
Results: CMS induced a significant decrease in sucrose intake, consistent with a depressive-like phenotype. Repeated ketamine administration reversed this behavioral effect. Most miRNAs were detected in serum, but only miR-182 showed significant expression changes, as determined by two-way ANOVA. Notably, miR-182 was upregulated in non-stressed ketamine-treated animals and the stressed ketamine and saline groups. This suggests a dual, context-dependent role of miR-182 in both susceptibility and antidepressant response.
Conclusions: Our findings support the hypothesis that miR-182 plays a context-sensitive role in depression-related molecular processes. In WKY rats, upregulation of miR-182 under stress may be associated with impaired PI3K/Akt signaling. In contrast, ketamine-associated elevation of miR-182 may contribute to neuroplasticity by targeting PTEN, a known inhibitor of Akt. This dual mechanism positions miR-182 as a promising biomarker and potential mediator of ketamine's antidepressant action.
Acknowledgements: The study was funded by Statutory Activity of the Department of Pharmacology, Maj Institute of Pharmacology PAS.

P.1.9. Machine Learning-Guided Discovery of Novel AChE and BChE Inhibitors for Alzheimer's Disease Treatment

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Background: Alzheimer's disease (AD) remains a critical global health challenge, partly driven by the loss of cholinergic function due to the enzymatic breakdown of acetylcholine by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). This leads to cognitive decline, as acetylcholine is vital for memory and learning. While FDA-approved cholinesterase inhibitors like Tacrine and Rivastigmine offer symptomatic relief, their effects are limited and often accompanied by adverse side effects. No new cholinesterase-targeting drugs have been approved in over two decades, highlighting the urgent need for novel therapeutic strategies with improved efficacy, selectivity, and safety.
Materials and Methods: Ondansetron, commonly prescribed to treat chemotherapy-induced nausea, has shown potential as a cholinesterase inhibitor and was selected for comparison with Tacrine and Rivastigmine. Using our in-house AI-based drug discovery platform (i-TripleD), the compounds were evaluated computationally for binding affinity, blood-brain barrier permeability, and solubility. A large-scale virtual screening of libraries from Enamine and MolPort was then performed. Generative AI modeling was applied to expand the chemical space and design novel analogues with potentially enhanced inhibitory properties. Top candidates were prioritized based on predicted binding affinity and ADME-Tox profiles for further experimental validation.
Results: Ondansetron showed the highest predicted binding affinity at 8.54 kcal/mol, compared to 7.44 kcal/mol for Tacrine and 6.38 kcal/mol for Rivastigmine. Experimentally, its IC_{50} values were 33 µM for AChE and 2.5 µM for BChE, indicating moderate to strong inhibition. These findings support its superior inhibitory potential over the FDA-approved drugs. Based on these results, AI-assisted screening identified several novel analogues with comparable or improved binding affinity and favorable pharmacokinetic properties, selected for further *in vitro* testing, including IC_{50} determination.
Conclusion: Ondansetron outperformed Tacrine and Rivastigmine in both computational and experimental assays. AI-guided screening successfully identified promising analogues that will undergo *in vitro* validation, offering a path toward developing next-generation cholinesterase inhibitors for AD treatment.
Acknowledgements: We thank the Sven and Lilly Lawski Foundation, the Swedish Research Council (VR), and NAISS/NSC for funding and computational support.

P.1.10. Mechanisms of anti-glioblastoma effect of 5-spirohydantoin derivative AR5 - in vitro study in human glioblastoma U87MG cell line

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Background: Glioblastoma multiforme (GBM) is one of the most dangerous types of cancer and remains hardly curable. Since recent reports suggest a significant contribution of dopamine signalling to glioblastoma, we screened a series of eight 5-spirohydantoin derivatives with diversified D2R and D4R affinity for their potential anti-GBM effects. Among them, the AR5 was selected as the most efficient, and in this study, intracellular mechanisms of its anti-GBM effects and its potential synergism with temozolomide (TMZ) were investigated.
Materials and Methods: The apoptotic (caspase-3 activity) and necrotic (number of necrotic cells, cathepsin D activity) markers were used to search for mechanisms of cell damage induced by AR5 in U87MG cells. Various inhibitors of intracellular pathways were used in mechanistic studies with AR5 and combined treatment with TMZ at the level of cell viability (MTT reduction test) and cytotoxicity (LDH release test). Moreover, the expression of D2-like receptors (D2R, D3R, and D4R) was measured in U87MG by Simple Protein Jess method.
Results: D2R, D3R, and D4R are expressed in U87MG cells. The cell damaging effects of AR5 in U87MG cells were connected with an increase in necrotic but not caspase 3-dependent apoptotic cell death markers. The induction of oxidative stress, apoptosis, necroptosis, ferroptosis, cathepsin D, ATM, JNK, p38, PI3K/Akt, and ERK1/2 kinase was excluded from the AR5-mediated anti-GBM effects. However, calpain inhibitor, calpeptin, and mTOR inhibitor, rapamycin, exaggerated the AR-5-mediated cell damage in U87MG cells. In addition, AR5 significantly increased the cell damage induced by TMZ. Based on the associative analysis between the anticancer activity of AR5 in U87MG, its affinity to D2R or D4R, and taking into account the expression level of D2R and D4R in this cell line, we rather excluded a significant contribution of antagonism to D2R or D4R from the anticancer activity of this compound.
Conclusions: The AR5-mediated anti-GBM effect is associated with the induction of necrotic but not apoptotic cell death and seems not to be connected with D2R or D4R antagonism. The combination of AR5 with TMZ, calpain, or mTOR inhibitors appears to be reasonable in increasing anticancer effects in GBM.
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P.1.11. Pharmacokinetics of inhaled Esketamine in rat and dog plasma

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Background: Esketamine, a dissociative anaesthetic, is under development as an inhaled therapy for treatment-resistant bipolar depression. Understanding the pharmacokinetics (PK) of esketamine and its principal metabolites—noresketamine (NKT) and hydroxynorketamine (HNK)—is critical for assessing systemic exposure, safety, and the translational relevance of preclinical models. This poster presents an evaluation of the PK of esketamine and its major metabolites in Wistar Han rats and Beagle dogs following repeated inhalation of esketamine.
Materials and Methods: Wistar Han rats and Beagle dogs received esketamine hydrochloride dry powder formulation by nose-only inhalation for 120 minutes, twice weekly. Plasma concentrations of esketamine, NKT, and HNK were measured by LC/MS/MS after dosing on Day 1 and Day 179 for rats; and on Days 1, 88, 176, and 267 for dogs. Key PK parameters, including C_{max}, t_{max}, $t_{1/2}$, sex ratios, and metabolite-to-parent ratios, were determined using WinNonlin 8.4.
Results: In rats, systemic exposure to esketamine (C_{max}, $AUC_{(0–t)}$) was higher in females than in males, with moderate accumulation observed after repeated dosing: C_{max} on Day 179 was up to 3.3-fold higher than on Day 1 in females, and $AUC_{(0–t)}$ on Day 179 was up to 2.4-fold higher than on Day 1 in females. Metabolite-to-parent ratios (HNK and NKT) were substantially higher in rats than in dogs, indicating species differences in metabolic pathways. In dogs, males generally exhibited higher esketamine exposure than females. Moderate accumulation was observed on Day 267: both C_{max} and $AUC_{(0–t)}$ were up to 2.1-fold higher in males. The terminal half-life of esketamine was shorter in dogs than in rats. No quantifiable analyte was detected in control or pre-dose samples on Day 1 in either species.
Conclusions: These studies provide a comprehensive comparative assessment of the pharmacokinetics of inhaled esketamine and its major metabolites in rats and dogs. Notable species and sex differences were observed in systemic exposure and metabolite formation, with rats exhibiting higher metabolite-to-parent ratios and a longer esketamine half-life. These findings inform the selection of preclinical models, dose translation, and safety assessment for the development of inhaled esketamine in treatment-resistant bipolar depression.

P.1.12. Age- and Sex-Dependent IgG *N*-Glycosylation Profiles from Blood and Saliva in the Turkish Population

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Background: This study investigates the age- and sex-related changes in the IgG N-glycome obtained from dried blood spot (DBS) cards in the Turkish population. By comparing the glycosylation features of IgG derived from saliva and blood, the study aims to explore population-specific variations and the concordance of salivary glycan traits with systemic profiles.
Materials and Methods: IgG was isolated from DBS cards and unstimulated saliva samples using protein G-sepharose beads. Blood-derived IgG N-glycans were released and labeled for hydrophilic interaction liquid chromatography with fluorescence detection (HPLC-HILIC-FLD). Nano-liquid chromatography tandem mass spectrometry (nLC-MS/MS) was used to characterize site-specific glycopeptides in both biofluids. Statistical analyses included age- and sex-stratified evaluations, Spearman correlation analyses were applied to blood IgG glycan profiles, and identification of significantly changing glycan traits across sex and age groups. Differential glycan abundance was assessed using non-parametric tests with multiple testing correction to detect sex-specific glycosylation signatures.
Results: The analysis revealed distinct age- and sex-associated shifts in IgG glycosylation in DBS-derived samples, including decreased galactosylation and sialylation with age. Comparative analysis demonstrated a moderate to high correlation between salivary and blood-derived IgG glycoforms, especially for major glycan structures. Notably, sex-specific glycan differences were identified, with certain structures being significantly more or less abundant in male versus female participants.
Conclusions: The findings support the feasibility of using DBS cards for large-scale population studies on IgG glycosylation and provide evidence for salivary IgG glycome as a reflective surrogate of systemic glycosylation patterns. The results highlight age- and sex-dependent differences in IgG glycosylation within the Turkish population and suggest potential for minimal-invasive glycomic profiling in personalized ageing diagnostics.
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P.1.13. Physiochemical Alterations in Neuronal Cell Membranes Induced by SSRIs: Correlation with Behavioral Studies

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Background: The brain's response to psychotropic drugs extends beyond biochemistry. Biophysical properties like tissue stiffness are critical for synaptic plasticity and neuronal health, yet they remain an underexplored aspect of pharmacology. This study investigates whether SSRIs alter the brain's nanomechanical architecture in addition to their known biochemical effects. Using Atomic Force Microscopy (AFM), we map these changes to uncover a new dimension of drug action and gain insight into the physical basis of learning. Understanding such biophysical effects could aid in designing safer, more effective therapeutics.
Materials and Methods: C57Bl/6J mice were treated for 28 days (i.p.) with fluoxetine (10 mg/kg), escitalopram (2 mg/kg), or vehicle (water). Spatial memory and learning strategies were assessed using the Modified Barnes Maze (MBM). Young's modulus of the cortex and hippocampus was determined using AFM in PeakForce QNM mode. Measurements were performed in liquid to mimic physiological conditions, allowing for precise determination of Young's modulus in the kPa range.
Results: Fluoxetine treatment impaired spatial learning, which was associated with increased use of a random search strategy and a modest rise in tissue stiffness in both the cortex and hippocampus. In contrast, escitalopram did not impair learning and promoted an efficient spatial strategy during recall. This positive behavioral profile was, however, accompanied by a profound increase in tissue stiffness, especially in the cortex, with a lesser change in the hippocampus.
Conclusions: Our findings, using Atomic Force Microscopy, reveal a hidden mechanical dimension to SSRI action. We show that a drug's cognitive effects can be decoupled from its impact on tissue mechanics. The case of escitalopram, which improved learning strategy despite inducing dramatic cortical stiffening, highlights this complexity. This establishes nanomechanical mapping as an essential methodology for uncovering the full biological impact of neuroactive drugs. By 'feeling' the brain's response at the nanoscale, AFM provides a missing piece of the puzzle, paving the way for a better understanding of drug efficacy and the development of safer therapies.
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P.1.14. Behavioural and pharmacological evaluation of the psilocybin analogue baeocystin in Wistar rats

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Background: Baeocystin is a naturally occurring tryptamine-based compound found in various psychoactive mushrooms, including several species of *Psilocybe genus*. Due to its structural similarity to psilocybin, which has shown a therapeutic potential in treatment of psychiatric disorders, there is a growing interest in investigating whether baeocystin exhibits comparable effects. This study investigated the pharmacokinetic profile and acute behavioural effects of baeocystin in Wistar rats.
Materials and Methods: Behavioural assessments, including locomotor activity and its spatial characteristics (in the open field test) and sensorimotor gating measured by prepulse inhibition were evaluated after subcutaneous administration of 1.25 or 5 mg/kg baeocystin. Pharmacokinetics and brain-serum ratios were analyzed after the 5 mg/kg sc. dose.
Results: Pharmacokinetics demonstrated that both baeocystin and its metabolite, norpsilocin, have a very limited ability to cross the blood-brain barrier. Consistent with the pharmacokinetic profile, baeocystin had no significant effects on locomotor activity, exploratory behaviour, anxiety-like responses, or sensorimotor gating at doses of either 1.25 or 5 mg/kg.
Conclusions: Our results suggest that baeocystin has minimal to no behavioural effects in rats, probably due to its poor permeability across the blood-brain barrier. This limited penetration may account for its negligible neurobiological and psychedelic activity.
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P.1.15. Development of a Multiparametric Screening Platform for Functional Evaluation of RdRp Inhibitors as Antiviral Therapeutics Against SARS-CoV-2

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Background: The COVID-19 pandemic highlighted the urgent need for rapid and scalable strategies to identify effective antiviral compounds. Targeting the RNA-dependent RNA polymerase (RdRp) complex of SARS-CoV-2, we developed and applied an integrated drug discovery platform combining biophysical binding assays, high-throughput biochemical screening, and cellular activity profiling. This platform enables systematic identification and validation of RdRp inhibitors across the hit-to-lead continuum.
Materials and Methods: A curated library of 280 compounds was screened for direct interaction with recombinant RdRp using Surface Plasmon Resonance (SPR), allowing real-time affinity profiling. Hit structures were assessed in a time-resolved FRET-based assay (TR-FRET) for RdRp enzymatic inhibition. To bridge biochemical findings with functional relevance, we engineered a luciferase-based cellular reporter system that represents the full RdRp complex (nsp12/7/8) to quantify intracellular polymerase activity in live cells.
Results: Out of 30 prioritized compounds—including remdesivir, molnupiravir analogs, and proprietary nucleoside derivatives—26 demonstrated RdRp inhibition in the TR-FRET assay. However, only remdesivir achieved functional inhibition above the defined threshold (>30%) in the cellular assay, indicating that biochemical activity alone is insufficient to predict intracellular efficacy.
Conclusions: Our results highlight the importance of orthogonal validation pipelines in antiviral drug development. The established platform enables mechanistic deconvolution of candidate inhibitors and provides a versatile framework for iterative compound optimization. This work contributes to the convergence of bioanalytical technologies, synthetic chemistry, and functional genomics, accelerating the discovery of antiviral agents with clinical translation potential. Moreover, the modular design of this approach creates opportunities for future integration with AI-driven bioinformatics workflows in diverse areas, such as oncology and neuropharmacological applications.
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P.1.16. Regulation of hepatic cytochrome P450 by hypothalamic NMDA receptors: The role of PVN and ARC nuclei

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Background: Cytochrome P450 (CYP) is a superfamily of hemoprotein enzymes involved in the metabolism of endogenous substrates and xenobiotics, including drugs. Our previous study demonstrated that CP-101,606, an antagonist of the GluN2B subunit of the NMDA receptor, negatively affected hepatic cytochrome P450 expression and activity following intraperitoneal administration. The aim of the present study was to investigate the effect of intracerebral administration of CP-101,606 into the paraventricular or arcuate nuclei of the hypothalamus on the central neuroendocrine regulation of cytochrome P450.
Materials and Methods: The experiment was conducted on male Wistar rats. Guide cannulas were bilaterally implanted into either the paraventricular (PVN) or arcuate (ARC) nuclei of the hypothalamus. CP-101,606 was administered bilaterally into the PVN or ARC at a dose of 3 µg, once daily for five consecutive days. Cytochrome P450 activity in liver microsomes was assessed based on the velocity of specific metabolic reactions. CYP protein levels in liver microsomes were determined by Western blotting, mRNA levels by qRT-PCR, and hormone concentrations in the serum, pituitary, and hypothalamus by ELISA.
Results: Repeated administration of CP-101,606 into the PVN led to increased somatostatin levels in both the PVN and pituitary, accompanied by decreased serum concentrations of growth hormone (GH) and corticosterone, and an elevated level of triiodothyronine (T_3). These hormonal alterations were associated with reduced expression (at both mRNA and protein levels) and diminished activity of hepatic CYP1A1/2, CYP2A1/2, CYP2B1/2, CYP2C11, and CYP3A enzymes. In contrast, repeated injections of CP-101,606 into the ARC resulted in decreased GH-releasing hormone levels in the ARC and pituitary, along with lower serum GH and corticosterone levels, while thyroid hormone concentrations remained unchanged. These changes were linked to decreased expression and activity of hepatic CYP1A1/2 and CYP2C11, as well as a reduction in *CYP3A2* mRNA level.
Conclusions: The results underscore the important role of NMDA receptors in the PVN and ARC in regulating hepatic CYP enzymes. They also show that CP-101,606 has a suppressive effect on the central neuroendocrine regulation of CYP expression, which should be considered when developing new drugs targeting the GluN2B subunit of the NMDA receptor.
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P.1.17. Neurodevelopmental and Behavioral Consequences of Maternal Western Diet in Rat Offspring

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Background: The increasing prevalence of neurodevelopmental disorders, such as autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD), highlights the critical role of early-life environmental factors. Among these, maternal nutrition has gained attention as a modifiable risk factor. Diets high in fats, sugars, and caseins, characteristic of the Western diet (WD), have been implicated in disrupting typical brain development and behavior in offspring. However, sex-specific effects of maternal WD on offspring behavior remain unclear.
Materials and Methods: Female Wistar Han rats were fed either a control diet (CD) or WD for 14 weeks, covering gestation and lactation. After weaning, all offspring received CD. Behavioral tests were conducted on postnatal days (PND) 30, 60, and 90, including locomotor activity (LA), novel object recognition (NOR), marble burying (MB), open field (OF), self-grooming (SG), novelty-suppressed feeding (NSFT), and elevated zero maze (EZM).
Results: Male offspring exposed to maternal WD showed increased body weight and adiposity; WD females showed increased adiposity without significant weight gain. Blood glucose levels were unaffected. In OF, WD males exhibited higher locomotor activity at PND 30 and 60, normalizing by PND 90; WD females showed a slight increase at PND 30. SG testing revealed prolonged grooming in WD males at PND 30 and reduced latency at PND 60 and 90, with no differences in females. In EZM, WD females showed more head dips at PND 30 and increased time in open arms at PND 90. Male CD offspring showed more stretched-attend postures at PND 60, and WD males had increased total arm entries at PND 90. NSFT, MB, LA and NOR tests showed no significant differences between diet or sex groups at any time point.
Conclusions: Maternal Western diet intake during pregnancy and lactation results in marked behavioral alterations in offspring, especially among males. Disturbances in exploratory behavior and anxiety-like responses were detected through OF, SG, and EZM assessments. These outcomes underscore the crucial impact of maternal nutrition on offspring neurodevelopment.
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P.1.18. Proneurogenic and immunomodulatory potential of mesenchymal stem cells in a murine model of temporal lobe epilepsy: phase-integrated therapeutic profiling

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Background: Temporal lobe epilepsy (TLE) is the most common form of epilepsy, accounting for approximately 60% of all cases. It is often resistant to pharmacotherapy and marked by chronic neuroinflammation and progressive neuronal remodeling. Mesenchymal stem cells (MSCs), due to their immunomodulatory and neuroregenerative properties, are increasingly investigated as therapeutic agents capable of modulating epileptogenic processes.
Materials and Methods: We used a dual experimental approach combining *ex vivo* and *in vivo* methods. Organotypic hippocampal cultures (OHCs) from epileptic mice were treated with MSCs-conditioned medium to assess secretome effects during the latent phase. In the chronic phase, MSCs were administered intracerebroventricularly. Therapeutic effects were assessed through behavioral testing, EEG recordings, and postmortem analyses, including biochemical assays and high-dimensional morphometric evaluation of hippocampal tissue.
Results: MSCs secretome enhanced expression of nestin and doublecortin, reduced NF-κB (p50/p105) and LDH levels, and modulated IL-6 release in OHCs. *In vivo*, transplanted MSCs were selectively localized to epileptic foci and remained viable for five weeks. Treatment reduced microgliosis and astrocytic hypertrophy, exerted immunomodulatory effects, and significantly shortened spontaneous seizure duration. EEG indicated improved neural network balance restoration.
Conclusions: MSCs and their secretome modulate cellular and molecular features of epileptogenic tissue in a phase-dependent manner. These findings indicate that MSCs-based interventions may modulate inflammatory responses and promote neuroregeneration, thereby influencing the course of epileptogenesis.
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P.1.19. Coadministration of Scopolamine and mGlu2 NAM VU6001966 as a Novel Antidepressant Approach: Rat Frontal Cortex Neurochemistry and Behavior

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Background: Conventional antidepressants typically require weeks of daily dosing to achieve a full response. In contrast, clinical studies provide evidence that scopolamine, a non-selective muscarinic receptor antagonist, can induce rapid and potent antidepressant effects within a few days of treatment. However, the side effects of scopolamine remain a concern. To address this issue, we have investigated the effect of a single acute coadministration of scopolamine and a negative allosteric modulator of the mGlu2 receptor, VU6001966, on rat behavior using a forced swim test (FST) and locomotor activity test. The effect of given compounds on the extracellular levels of neurotransmitters in the rat frontal cortex (FCX) was examined using microdialysis in freely moving rats.
Materials and Methods: The FST test included two sessions: a pretest to habituate rats to water for 15 minutes (one day before drug administration), and a main test to measure the total duration of immobility over a 5-minute period (45 minutes after drug administration). To assess neurotransmitter levels, including dopamine, serotonin, glutamate, and GABA, microdialysis was performed in the FCx of freely moving rats.
Results: Both scopolamine and VU6001966 induced dose-dependent antidepressant-like effects in the FST test without affecting locomotor activity. Furthermore, VU6001966 enhanced extracellular dopamine level while lowering glutamate, without affecting GABA level. Both scopolamine alone or in combination with VU6001966 increased dopamine and glutamate levels in the FCX, without affecting GABA levels.
Conclusions: Our results suggest that coadministration of scopolamine with mGlu2 NAM might be a promising alternative to using scopolamine alone in depression therapy, potentially allowing for a lower therapeutically effective dose. The common mechanism underlying the observed behavioral effects of the tested drugs may be associated with the modulation of the glutamatergic and dopaminergic systems.
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P.1.20. Physicochemical Characterization of Ti6Al4V–Hydroxyapatite Composites Produced by Powder Metallurgy for Bone Regeneration

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The demographic shift toward an aging population is driving a significant increase in the demand for orthopedic implants, particularly for hip and knee arthroplasty. To address the clinical requirement for long-term implant durability and to minimize the incidence of revision surgeries, there is an ongoing need to develop advanced biomaterials with enhanced mechanical and biological performance.
This study focuses on the fabrication and characterization of composite materials based on the Ti6Al4V titanium alloy and hydroxyapatite (HAp) for applications in bone tissue regeneration. Hydroxyapatite, a bioactive ceramic, was synthesized via a controlled wet precipitation method. The resulting HAp powders were subjected to comprehensive physicochemical characterization, including phase identification and purity assessment, confirming successful synthesis.
Ti6Al4V/HAp composites were produced using conventional powder metallurgy techniques, followed by sintering. The microstructure and physicochemical properties of the composites were evaluated using scanning electron microscopy with energy-dispersive spectroscopy (SEM-EDS), optical microscopy, surface roughness measurements, particle size analysis (PSA), X-ray fluorescence (XRF) and thermal analysis (simultaneous measurement of mass changes (TG) and thermal effects (DSC)). The bottom-up approach enabled precise control over the morphology of HAp particles and ensured their homogeneous distribution within the titanium alloy matrix.
The incorporation of hydroxyapatite into the Ti6Al4V matrix is intended to impart bioactivity to the composite, thereby promoting osseointegration and potentially reducing the risk of implant loosening. While further in vitro and in vivo biological evaluations are necessary, the synthesized Ti6Al4V/HAp composites exhibit promising physicochemical properties that warrant continued investigation for orthopedic implant applications.
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P.1.21. Maternal Monosaccharide Diets Modulate Hippocampal Neurogenesis in Rat Offspring

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Background: The hippocampus, essential for emotional regulation and neurogenesis, is highly susceptible to nutritional imbalances. Recent studies have highlighted that maternal monosaccharide diets—glucose (GLU) or fructose (FRU)—can dysregulate the emotional status of offspring (Witek, 2023). However, the effects of maternal monosaccharide consumption on hippocampal neurogenesis in offspring are still unclear. Therefore, this study investigates the impact of maternal GLU or FRU diets on markers of hippocampal neurogenesis in rat offspring.
Materials and Methods: Wistar dams were fed standard (SD), GLU, or FRU diets during pregnancy and lactation. Next, the ventral and dorsal hippocampal (vHIP and dHIP) regions were dissected from both male and female offspring rats following decapitation at postnatal days 28 and 63 (PND28 and PND63). Molecular analyses were conducted using RT-qPCR to assess the expression of the *Gfap*, *Rbfox3*, *Bdnf*, *Nes*, and *Dcx* genes using TaqMan® Gene Expression Assays. Additionally, the protein levels of selected targets—GFAP, NeuN, and DCX—were quantified using ELISA.
Results: Maternal GLU and FRU diets increased the expression of *Bdnf* and *Dcx*, while FRU specifically upregulated *Rbfox3* in the dHIP of male offspring at PND28. Conversely, in the vHIP of males at PND63, both GLU and FRU diets increased *Gfap* expression. At the same time, FRU selectively increased *Dcx* and *Rbfox3* expression in males and significantly upregulated *Bdnf* in female offspring. Neurochemically, maternal GLU or FRU diets decreased protein levels of GFAP, NeuN, and DCX in the vHIP of male offspring at both PND28 and PND63. Furthermore, in the dHIP, GLU and FRU diets either decreased or increased the levels of GFAP, NeuN, and DCX proteins in males at PND28 and PND63, respectively.
Conclusions: Our results suggest that maternal monosaccharide diets, particularly the FRU diet, may influence markers of hippocampal neurogenesis in offspring. These changes appear to be specific to sex, age, and region, indicating that maternal nutrition can differentially affect neurogenic processes across distinct hippocampal regions. Moreover, these molecular findings support our previous behavioral observations, offering further insight into the potential mechanisms through which maternal diet may influence neurodevelopmental alterations.
Acknowledgements: This work was supported by the Polish National Science Centre (Kraków, Poland) research grant no. UMO-2023/49/N/NZ1/03606 to Witek K.

P.1.22. Glucocorticoid receptor-regulated genes link stress with brain-related human phenotypes

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Background: Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of glucocorticoids and subsequent activation of the glucocorticoid receptor (GR), a transcription factor that regulates genes involved in metabolism, immunity, and brain function. Chronic HPA activation, which is considered a hallmark of stress-related disorders such as depression, disrupts GR signaling. However, GR-regulated genes in the human brain remain poorly characterized, limiting our understanding of stress-related pathomechanisms.
Materials and Methods: We created a database of GR-dependent genes from 51 publications, including 54,503 records and 13,251 unique genes, limited to protein-coding entries according to biomaRt v110. Based on this, we defined 26 GR-dependent signatures (14 downregulated and 12 upregulated), each comprising the 50 top-ranked genes with the most pronounced expression changes, representing various tissues and cell types. Next, two metasignatures were derived, consisting of genes showing the most consistent and robust differential expression across all tissues. The metasignatures, together with brain-derived signatures, were tested for genetic associations with 54 mental health phenotypes from Genebass (SKAT, $p < 0.05$).
Results: Among 189 GR-dependent genes tested, 125 (66%) showed significant associations with 54 mental health phenotypes, resulting in 955 potential gene–phenotype links (FDR < 0.2). Several GR-induced transcripts in the brain, including *RHOU*, *RAMP2*, and *LMOD1*, showed strong associations ($p < 1 \times 10^{-5}$) with the depression-related phenotype "recent changes in speed/amount of moving or speaking" (ID:20518). These genes are involved in vascular remodeling, actin cytoskeleton regulation, and cellular signaling. *IL1B*, an immune system mediator affecting blood–brain barrier integrity and neuronal excitability, present in the metasignature of downregulated genes, was also linked to the same phenotype.
Conclusions: Although GR-dependent genes are involved in diverse biological processes, our analysis identifies a subset strongly associated with depression-related traits and linked to cerebral blood flow and vascular function. These findings suggest that GR activity may contribute to mood disorders, in part through cerebrovascular mechanisms.
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Other topic

P.1.23. Delving the Potential Transcriptional Role of Glutaminase GLS2 in Glioblastoma Cells Through a Transcriptomic Approach

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Background: Glioblastoma (GBM) is the most aggressive primary brain tumor, with limited therapeutic options. GBM cells rely on glutamine (Gln) metabolism for energy. Gln is catabolized by two enzymes encoded by *GLS* and *GLS2*. *GLS*, whose products promote tumorigenesis, is overexpressed in GBM, whereas *GLS2* is silenced. Reintroduction of *GLS2 in vitro* suppresses GBM cell proliferation and viability. Nuclear localization of GLS2 reported in some studies suggests a potential transcriptional role for GLS2, although evidence remains limited.
Materials and Methods: U251MG GBM cells were transfected with either the full-length *GLS2* transcript or an empty vector (pcDNA). GLS2 level and its activity were assessed by western blot and enzymatic assay, respectively. Furthermore, cell viability, doubling time and ability to form colonies were assessed. Then, RNA sequencing was performed.
Results: Western blot confirmed the presence of GLS2 in *GLS2*-transfected cells, for which glutaminase activity was increased and viability was decreased. Compared to the controls, *GLS2*-transfectants showed 1432 differentially expressed genes (DEGs): 994 upregulated and 438 downregulated. Notably, two upregulated genes, *FOSL2* (encoding FOSL2 transcriptional factor) and *CD274* (encoding PD-L1), are linked to immune escape mechanisms, with PD-L1 previously proposed as an indirect target of GLS2. *FOSL2* was also predicted *in silico* to regulate 344 DEGs. Functional enrichment analysis of all DEGs or upregulated DEGs revealed significant involvement in pathways related to cell adhesion, motility, and morphogenesis. In contrast, downregulated genes were associated with only two marginal pathways.
Conclusions: Overexpression of *GLS2* decreased viability and proliferation of U251MG GBM cells and altered expression of several genes. The predominance of upregulated genes suggests a transcriptional regulatory role for GLS2, primarily through gene activation. Based on pathway enrichment analysis we conclude that the genes correlated with tumorigenesis are found mainly among the upregulated ones, while downregulated genes seem to be irrelevant in this context. Although *in vitro* studies have shown suppressive phenotypic effects of GLS2, gene expression data do not support these findings. The enrichment of immune escape pathways observed in *GLS2*-transfected cells merits further investigation.
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P.1.24. Comparison of machine learning models for predicting pharmacotherapy in obsessive-compulsive disorder

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Background: Obsessive-compulsive disorder (OCD) is a complex neuropsychiatric disorder, and accurate diagnosis and optimal pharmacotherapy selection pose serious clinical challenges. The advent of artificial intelligence (AI) and bioinformatics applications in medicine has led to a proliferation of opportunities for the utilization of machine learning (ML) in therapeutic decision-making. This project aims to implement a selection of AI models to predict the pharmaceutical classification assigned to patients diagnosed with OCD.
Materials and Methods: The clinical, psychiatric, and demographic data of patients diagnosed with OCD were analyzed. The variables included subtypes of obsessions and compulsions, Y-BOCS scale scores, comorbid mood and anxiety disorders, and participants' age, gender, and level of education. Feature engineering, including the creation of composite variables and age categorization, as well as coding of categorical data, were carried out. Classification models, such as logistic regression, Random Forest, XGBoost, and LightGBM, were employed.
Results: A comparison of the models indicated discrepancies in pharmacotherapy prediction performance depending on the employed algorithm. The purpose of this poster is to demonstrate the disparities in the ability to identify clinical patterns, which consequently result in the selection of specific drug groups. The most significant predictors of treatment were identified using a feature validity analysis.
Conclusions: Machine learning is being used in neuropsychiatry to support therapeutic decision-making. Comparing ML models emphasises the importance of selecting the right algorithm and ensuring the quality of the input data. The project confirms the validity of combining bioinformatics approaches with AI to develop personalized clinical decision support tools in psychiatry.
Acknowledgements: The project is carried out as a self-directed work. The author expresses gratitude to the research mentors and the open-source community for the development of available analytical tools and machine learning libraries.

P.1.25. Evaluation of the effects of ACEA 1021 (NMDA receptor antagonist) on Glial Fibrillary Acidic Protein in dexamethasone-induced neurotoxicity

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Background: Chronic elevation of circulating glucocorticoids (GCs) levels causes neuronal degeneration of hippocampal pyramidal neurons and striatum. GCs potentiate stress or ischemia-induced accumulation of excitatory amino acids in the hippocampal extracellular space, facilitating glutamate/Ca^{2+} cascade and endangering hippocampal neurons.
ACEA 1021 (licostinel), a selective glycine site antagonist of the NMDA receptor complex, prevents the excitotoxic action of high extracellular glutamate levels. Recent studies revealed GFAP (Glial Fibrillary Acidic Protein) functions in astrocytes, including migration, blood barrier functioning, signal transduction, and neuron-glia interactions. CNS injuries like cerebral ischemia cause significant depression in hippocampal slices lacking GFAP, triggering pyramidal neuron loss in mice. Neuronal death increases when GFAP is absent in injury conditions.
Materials and Methods: This immunohistochemical study evaluated ACEA 1021's neuroprotective effect on GFAP in the hippocampal CA3 subfield using a dexamethasone-induced neurotoxicity model in mice. Neurotoxicity was induced by dexamethasone (DEX, synthetic GC receptor agonist) administration to albino mice at 16 mg/kg/day for 14 days.
ACEA 1021 was administered at doses of 1.25, 2.5, and 5.0 mg/kg/day intraperitoneally, 15 minutes before DEX daily. 48 hours after the final injection, mice were anesthetised and brains examined immunohistochemically using Monoclonal Mouse Anti-Human Glial Fibrillary Acidic Protein. The ratio of immunopositive cells to total cells (average %) in the hippocampal CA3 subfield was determined, along with average immunoreaction intensity [8-bit grey scale] on GFAP in the pyramidal layer of the hippocampal CA3 subfield.
Results: Analysis showed significant differences in GFAP-positive cell content on hippocampal cross-sections and immunohistochemical reaction intensity in the DEX group compared to controls, indicating a semi-quantitative increase in labelled protein. ACEA 1021 administration (particularly at 2.5 and 5.0 mg/kg) increased GFAP immunohistochemical reaction intensity in DEX-treated animal groups.
Conclusion: Results suggest significant GFAP involvement in protecting hippocampal pyramidal neurons from damage caused by chronic dexamethasone administration, potentially serving as a marker for such damage. Further study is required to elucidate ACEA 1021's effect on GFAP in GC-induced neurotoxicity models.
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P.1.26. 3D printing of double-crosslinked chitosan hydrogels modified with phosphate-silicate-borate glass

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Background: One of the rapidly developing areas of biomedical engineering is the design of hydrogel materials based on natural polymers. Their composites with bioactive glasses capable of stimulating bone and cartilage regeneration processes represent promising scaffolds for tissue engineering. The ability to tailor their properties and the application of 3D printing technology significantly expand their potential use, including matrices for cell culture.
Materials and Methods: This study focused on developing chitosan-based composite hydrogels modified with phosphate-silicate-borate glass (P_2O_5–SiO_2–B_2O_3–CaO–MgO). Dextran functionalized with aldehyde groups served as the primary crosslinking agent. After optimizing hydrogels compositions, the most promising materials were selected for 3D printing using the Cellink BIO X bioprinter. A comprehensive range of analyses was conducted, including scanning electron microscopy with EDS, rheological measurements (assessing time and frequency dependencies, and viscosity), in vitro bioactivity testing, degradation studies based on mass loss, and evaluation of the stability of 3D-printed hydrogel scaffolds during incubation in simulated body fluid (SBF).
Results: The results demonstrated that the incorporation of P-Si-B bioactive glass into the chitosan-dextran hydrogel significantly altered its chemical, physical, and functional properties. Bioglass incorporation improves phosphate formation, which leads to high bioactivity of materials. The incorporation of bioglass significantly influenced the rheological properties by reducing the crosslinking time and enhancing both the initial viscosity and shear-thinning behavior. Furthermore, the presence of bioactive glass accelerated the degradation rate of hydrogel materials, while the stability of 3D printed hydrogel scaffolds modified with bioglass remained similar to that of the base materials.
Conclusions: The developed hydrogel composites exhibit promising characteristics for use in regenerative medicine, particularly in bone and cartilage tissue engineering. Their tailored printability, rheological behavior, self-healing capacity, and structural stability in physiological conditions support their potential for further in vitro and in vivo research.
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P.1.27. Aldehyde-Functionalized Dextran and Borate Glasses as Dual Cross-Linkers in Chitosan-Based Bio-Inks

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Background: In recent years, hydrogels made from chitosan have become increasingly utilized, especially within the realms of tissue engineering and 3D bioprinting. Chitosan, a natural polysaccharide obtained from chitin by deacetylation, is renowned for its biocompatibility, biodegradability, and gel-forming capabilities. These characteristics make it an appealing substrate for creating hydrogels. The functionality of chitosan-based hydrogels can be improved with specific cross-linking strategies. Aldehyde-functionalized dextran acts as an effective cross-linker by facilitating the formation of Schiff base bonds. Moreover, the addition of bioactive glasses provides a dual cross-linking system: one involving Schiff base bonds and another through dynamic ester bonds between the polymers and glass particles.
Materials and Methods: This study aimed to develop and optimize a chitosan-based bio-ink for 3D printing using the CELLINK Bio X bioprinter. The hydrogels were cross-linked with aldehyde-functionalized dextran and supplemented with borate bioactive glass of two oxide compositions: A2B40 (40 mol% B_2O_3, 54 mol% CaO, 6 mol% P_2O_5) and S2B80 (80 mol% B_2O_3, 16 mol% CaO, 4 mol% P_2O_5). Rheological properties and gelation times were evaluated. Scaffolds were incubated in Simulated Body Fluid (SBF), and their morphology and elemental composition were assessed by SEM/EDX. FTIR spectroscopy identified functional groups, while ICP-OES tracked elemental release.
Results: Borate bioactive glass significantly affected hydrogel behavior, reducing gelation time and enhancing rheological properties. FTIR confirmed Schiff base cross-linking by identifying characteristic absorption bands. ICP-OES enabled detailed elemental analysis after incubation in SBF. SEM/EDX showed well-preserved scaffold architecture and homogenous glass distribution in the hydrogel matrix.
Conclusions: The developed hydrogels exhibit great potential as bio-inks for 3D printing in tissue engineering. Dual cross-linking with functionalized dextran and borate glasses offers tunable gelation and printability, highlighting their applicability in regenerative medicine.
Acknowledgements: This work was supported by the National Science Centre, Poland (Grant no. 2023/49/N/ST11/02999) and the “Excellence Initiative – Research University” program at AGH University.

P.1.28. Disruption of FOXM1 activity impairs cancer stem cells in colorectal cancer

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Background: Colorectal cancer (CRC) is the third most common malignancy worldwide and a major cause of cancer-related mortality. Despite advances in treatment, many patients experience relapse or develop resistance to chemotherapy. One of the key contributors to these challenges is the presence of cancer stem cells (CSCs) - a subpopulation capable of self-renewal and driving tumor regrowth. The transcription factor FOXM1 is a critical regulator of cell proliferation and survival and plays an important role in maintaining CSC activity.
Materials and Methods: In this study, we developed small-molecule inhibitors DK1 and DK2, designed to block the DNA-binding domain of FOXM1, thereby disrupting its transcriptional activity. Molecular docking and TR-FRET assays confirmed that both compounds bind to key residues in the FOXM1-DNA interface. Their functional activity was tested in colorectal cancer cell lines (SW480, SW620) and compared with normal epithelial cells (CCD841).
Results: Treatment with DK1 and DK2 significantly reduced cancer cell viability and colony-forming ability, with minimal cytotoxic effects observed in non-cancerous cells. Notably, DK1 induced clear morphological changes in CRC cells, suggestive of cellular stress or differentiation, which were not evident in normal cells. Western blot analysis revealed a dose-dependent decrease in FOXM1 protein levels following treatment. Furthermore, both compounds outperformed 5-fluorouracil in inhibiting colony formation. Flow cytometry analysis of CSC markers (CD24, CD44, CD133, CD326) demonstrated alterations in CSC subpopulations, indicating that FOXM1 inhibition may directly affect CSC function.
Conclusions: These findings validate the DNA-binding domain of FOXM1 as a selective and potent therapeutic target in CRC. By simultaneously impairing tumor growth and CSC-driven resistance, DK1 and DK2 represent promising candidates for the development of next-generation targeted therapies.
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P.1.29. Light Meets Nature: Exploring Bio-Based Materials for DLP 3D Printing

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Digital Light Processing (DLP) is a form of 3D printing technology based on photopolymerization in the liquid state. A key component of the DLP process is the photocurable resin—a low-viscosity liquid composed of photoreactive monomers and/or oligomers, a photoinitiator, and additives that modify mechanical, optical, and application-specific properties.
With the growing emphasis on sustainability and reducing the environmental impact of manufacturing, there is increasing interest in replacing conventional resin components with natural-origin materials. Natural polymers such as lignin, starch, cellulose, chitin, and plant-based proteins possess chemical structures that can be modified for use in photopolymerization. Additionally, plant-derived oils—including soybean, castor, and linseed oil—can serve as precursors in the synthesis of photocurable acrylate or epoxy resins.
An important research direction involves the development of biohybrid resins, which combine renewable raw materials with small amounts of traditional photoreactive compounds to balance environmental sustainability with technical performance. Ongoing studies focus on adjusting viscosity, reactivity, and chemical stability, as well as optimizing interactions with UV/LED light sources used in DLP printers.
Integrating renewable feedstocks into DLP technology can also improve recyclability and safety, especially in medical or educational applications. However, challenges remain, such as limited thermal stability, yellowing, and volume shrinkage during curing, all of which require further technological refinement and material science innovation.
Main aspect discussed in this article is the synthesis and application of a novel monomer, -diamine-vanillin dimethacrylate. The effects of the newly synthesized monomer on the radical photopolymerization kinetics and the DLP 3D printing process were investigated. It has been proven that the addition of a biobased monomer to photocurable resins reduces induction time. This also proved the suitability of the biobased monomer for obtaining three-dimensional structures with improved mechanical properties. DVDMA bio-based monomers contain imine linkages that are easily cleaved under acidic conditions.
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P.1.30. The role of serum and sperm microRNAs in the intergenerational transmission of childhood trauma-related phenotypes

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Background: Childhood trauma has been associated with long-term behavioral and metabolic sequelae across generations. Emerging evidence from rodent studies supports a role for sperm RNAs in the intergenerational transmission of neuropsychiatric and metabolic disease susceptibilities through the patriline after early life stressful and traumatic experiences. However, the translational relevance of this concept in humans through the patriline remains understudied.
Materials and Methods: We systematically examined small RNAs in serum and sperm samples from different human trauma cohorts to synthesize evidence for the plausibility of intergenerational transmission of susceptibilities. These include Pakistani children (n=72, n=42 controls) and adult men (n=93) with histories of complex childhood trauma, as well as Bosnian families (n=22, n=20 controls) who lived through the genocide during their formative years. Small RNA sequencing (sRNA-seq) followed by RT-qPCR assays were performed on the collected serum and sperm samples from the Pakistani and Bosnian cohorts. Data was compared and correlated with neuropsychiatric scales and lipid profiles of the subjects intergenerationally. Additionally, we examined sperm from a mouse model of early life stress—unpredictable maternal separation and stress (MSUS) (n=6 MSUS, n=7 controls)—to assess whether microRNAs differentially regulated after childhood trauma in human sperm are similarly altered in a mouse model with transgenerational phenotypes.
Results: sRNA-seq revealed differential expression of 48 miRNAs in the serum of traumatized children vs. controls in Pakistan, whereas 29 miRNAs are altered in the sperm of trauma-exposed Pakistani men compared to controls. Two of these microRNAs, miR-145-5p and miR-223-3p, were also altered in the sperm of MSUS mice. Furthermore, some miRNAs were differentially expressed in the sperm of Bosnian men exposed to genocide. Importantly, neuropsychiatric symptoms in the Bosnian children correlated with miRNAs expression in the sperm of their fathers.
Conclusions: Collectively, these findings underscore the potential role of serum and sperm miRNAs in the intergenerational transmission of trauma-related phenotypes in humans and mice and support the candidacy of certain miRNAs as biomarkers of such effects.
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P.1.31. New Photoinitiators for 3D Printing Using One- and Two-Photon Photopolymerization in Medical Applications

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Background: Photopolymerization has a long history in the field of photochemistry. Due to its advantages, such as the absence of solvents and low energy consumption, it is widely used in the coatings, paint, and adhesives industries, as well as in medicine, in tissue engineering, treatment of skin diseases, and dentistry. This process mainly involves a chain reaction initiated by photoinitiators, which, upon exposure to light, generate free radicals or ions that start the polymerization. Modern photoinitiators are being developed to increase the efficiency of the process, improve material properties, and expand the range of applications. They are used in vat photopolymerization technologies such as SLA, DLP, TPP, CLIP, and LCD. These methods differ in curing speed, resolution, and material compatibility, with TPP offering the highest resolution. Additionally, it is crucial that new photoinitiators possess biocompatibility and bio-safety because 3D printing of micrometer-scale objects in medicine is an innovative technology enabling precise fabrication of microscopic structures that can be used for painless drug delivery through the skin.
Conclusions: The development of new photoinitiators and initiating systems is crucial for the future of 3D printing, particularly in medical, biotechnological, and sustainable material production applications. Many challenges remain in improving their efficiency, safety, and compatibility, requiring interdisciplinary research across chemistry, physics, and biology. Well-designed photoinitiating systems will drive the advancement of innovative 3D printing technologies.
Acknowledgements: Research funded under competition No. 2024/ABM/03/KPO/ project No. KPOD.07.07-IW.07-0125/24 entitled: “Project title: Luminescent theranostic compounds with anticancer activity, i.e., the combination of photodynamic therapy and diagnostics through imaging in a single molecule, and the development of 3D-printed local microneedle systems to ensure precise, individualized cancer therapy” from the National Plan for Recovery and Resilience, which is part of Investment D3.1.1 Comprehensive development of research in medical and health sciences, a project financed by the Medical Research Agency.

P.1.32. Neurochemical Modulation of Auditory Steady-State Responses: A Systematic Review

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Background: Gamma-range (γ, 30–100 Hz) auditory steady-state responses (ASSRs) are read-outs of cortical excitation–inhibition balance and are blunted in neuropsychiatric disorders. Neurochemical drivers remain unevenly mapped and confounded by numerous factors.
Materials and Methods: A PRISMA search (inception – Jun 2025) retrieved 70 eligible studies (25 human, 43 animal & 2 both). Each study was evaluated for neurochemical system, drug dose, post-dose interval, alertness state, recording technique, and stimulus design. Outcomes extracted were comparison of power, amplitude, synchronicity measures.
Results: System coverage: glutamatergic (Glu) 39, GABAergic (GABA) 28, cholinergic (ACh) 5, dopaminergic (DA) 5, serotonergic (5-HT) 4, cannabinoidergic 3, plus eight dual Glu–GABA reports.
Glutamate: High-affinity NMDA antagonists, such as MK-801, high-dose ketamine, or PCP consistently reduced 40 Hz phase-locking, while low-affinity memantine and low-dose ketamine or glycine-site modulators often enhanced synchrony. *GABA*: GABA_A potentiation followed an inverted-U curve: light sedative doses sharpened synchrony while deep doses abolished it; GABA_B agonists normalised Fragile X and autism-related deficits. *Acetylcholine*: Nicotinic agonism enhanced, and muscarinic blockade impaired, γ-ASSRs. *Dopamine*: D1 potentiation strengthened responses, whereas D2 antagonism produced context-dependent effects. *Serotonin & cannabinoids*: Psychedelic 5-HT2A agonists, serotonin reuptake inhibitors and Δ^9-THC uniformly suppressed ASSRs. Understudied systems (ACh, DA, 5-HT, endocannabinoid) accounted for < 20 % of experiments, indicating a major evidence gap. Harmonics, though reported only in 3 papers, often shifted more consistently than fundamentals, suggesting a more sensitive synchronicity biomarker.
Conclusions: Stable 30–100 Hz ASSRs require balanced NMDA-driven excitation and fast GABAergic inhibition, with acetylcholine, dopamine, serotonin and cannabinoids acting as dose- and state-dependent modulators. Reporting power, synchrony and harmonic strength together with dose, timing and vigilance enables coherent synthesis.
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P.1.33. Human iPSC-derived cardiomyocytes from Becker muscular dystrophy patients reveal disrupted iron homeostasis

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Background: Becker muscular dystrophy (BMD) is an X-linked recessive disorder caused by in-frame mutations in the *DMD* gene encoding dystrophin. Cardiomyopathy is the leading cause of death in BMD; however, its molecular basis remains poorly understood due to limited availability of animal models and patient-derived tissues. Human induced pluripotent stem cells (hiPSCs) offer a powerful platform for disease modeling, enabling the study of BMD in a patient-specific genetic context. This study aimed to generate patient-specific and CRISPR/Cas9-corrected BMD hiPSC lines to identify molecular signatures linked to cardiomyopathy.
Materials and Methods: Two BMD patients carrying in-frame deletions of exons 3–9 and 45–47 were included in this study. BMD hiPSCs were generated from peripheral blood mononuclear cells using Sendai vectors encoding OCT4, SOX2, KLF4, and c-MYC. CRISPR/Cas9-mediated homology-directed repair was used to correct *DMD* mutations and generate isogenic controls. All lines retained a normal diploid karyotype and expressed key pluripotency markers. Cardiomyocyte differentiation (hiPSC-CMs) was followed by RT-PCR and Western blot to confirm dystrophin restoration. Cytoplasmic and mitochondrial labile iron pools were assessed by flow cytometry using Calcein-AM and Rhodamine B probes. Expression of iron-regulatory genes and proteins was evaluated by qRT-PCR and Western blot.
Results: CRISPR/Cas9-mediated correction successfully restored full-length dystrophin expression in isogenic hiPSC-CMs. Compared to corrected lines, BMD hiPSC-CMs showed elevated cytoplasmic and mitochondrial iron levels. This iron overload was accompanied by reduced expression of *CISD1* (mitoNEET) and *SLC40A1* (ferroportin), and increased expression of *FTH1* (ferritin), *HAMP* (hepcidin), and *TFRC* (transferrin receptor), indicating widespread dysregulation of iron homeostasis. It was confirmed reduced mitoNEET protein level in BMD hiPSC-CMs.
Conclusions: BMD hiPSC-CMs exhibit dysregulated iron homeostasis, characterized by increased labile iron and altered expression of key iron-regulatory genes. These findings suggest that disturbances in iron metabolism contribute to the pathophysiology of BMD-associated cardiomyopathy and establish a platform for further therapeutic studies.
Acknowledgments: This study was supported by the MAESTRO grant from National Science Centre (NCN): 2018/30/A/NZ3/00412.

P.1.34. Between Law and Practice: Poland's Regulatory Deficit in iPSC Therapies and the Pharmacist's Untapped Potential

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Background: Induced pluripotent stem cells (iPSC) represent a major innovation in regenerative medicine and genetic disease treatment. Their clinical use is governed by complex regulatory frameworks. The European Union (EU) and Switzerland apply detailed but often restrictive rules, while the United States adopts a more flexible approach. Japan offers a balanced model that promotes innovation while ensuring safety. Poland, despite being part of the EU, remains significantly behind in the practical implementation of iPSC-based therapies.
Materials and Methods: A comparative analysis was conducted on the legal and regulatory frameworks governing iPSC therapies in the EU, USA, Japan, Switzerland, and Poland. The study included legislation, EMA/FDA/PMDA guidance, national procedures, ATMP registration, risk evaluation, donor consent, and genetic data ownership. Special attention was paid to the Hospital Exemption (HE) mechanism and the role of qualified professionals responsible for cell therapy production, control, and oversight.
Results: In the EU, iPSC therapies are regulated as Advanced Therapy Medicinal Products (ATMPs) under Regulation (EC) No 1394/2007. Countries like Germany and France operate expert bodies, functional HE systems, and reimbursement pathways. In contrast, Poland lacks operational procedures, oversight institutions, and public funding. The HE mechanism is not functional, and ATMPs are not reimbursed by the National Health Fund (NFZ). Clinical infrastructure and national coordination are lacking. A critical gap is the shortage of pharmacists trained in ATMP-related production, regulatory compliance, and quality assurance. Although pharmacists are ideally positioned to fulfill this role, only a few professionals in Poland currently possess these competencies.
Conclusions: Despite shared EU legislation, national implementation varies widely. Poland remains excluded from the real-world application of iPSC therapies due to the lack of expert bodies, financing pathways, and trained personnel. Developing a national strategy for ATMP implementation, especially by investing in the education and integration of advanced therapy pharmacists, is essential for enabling access to iPSC therapies in the Polish healthcare system.

P.1.35. Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes and Cardiac Progenitor Cells – Cell-Based Therapy in Murine Model of Myocardial Infarction

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Background: Cardiovascular diseases remain the leading cause of death worldwide, with ischemic heart disease frequently resulting in myocardial infarction and, ultimately, heart failure. In severe cases, heart transplantation remains the only effective option. Cell-based therapies aim to restore heart function post-infarction, but poor survival of transplanted cells limits their success. This study investigates the effect of transplantation of hiPSC-derived cardiomyocytes (hiPSCs-CM) and cardiac progenitor cells (hiPSCs-CPC).
Materials and Methods: hiPSCs expressing luciferase (Luc) were differentiated to hiPSC-CP (10 days) and hiPSC-CMs (21-25 days) using small molecules modulating the WNT pathway. Myocardial infarction was induced by LAD ligation in NOD/SCID immunodeficient mice. Immediately after the insult $5x10^5$ hiPSC-CMs or CPCs were injected into the hearts at the border of infarct area. Heart function was monitored with VEVO ultrasound, while cell engraftment by IVIS. Animals were observed for three months.
Results: Bioluminescence imaging 12 weeks after treatment demonstrated significantly higher engraftment of hiPSC-derived cardiomyocytes (hiPSC-CMs) compared to cardiac progenitor cells (CPCs).
Conclusions: Our findings confirm successful engraftment of hiPSC-derived cardiomyocytes (hiPSC-CMs) into cardiac tissue following myocardial infarction (MI) in a murine model. Injection of hiPSC-CPC was less efficient. Ongoing studies will evaluate improvements in heart function and the integration of transplanted cells with host myocardium.
Acknowledgements: This work was founded by SHENG-2 grant from the National Science Centre [2021/40/Q/NZ3/00165].

P.1.36. Tailored ALD coatings on NiTi alloy: physicochemical and biological evaluation for cardiovascular implant applications

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Background: Nickel-titanium (Nitinol) shape memory alloys are commonly used in cardiovascular implants such as ASD occluders and post-VAD closure plugs due to their superelasticity and shape memory properties. However, their high nickel content can lead to ion release under physiological conditions, posing cytotoxic and thrombogenic risks. To enhance biocompatibility, surface modification techniques are increasingly applied. Among them, atomic layer deposition (ALD) enables precise control of nanoscale coatings. By tailoring surface wettability (hydrophilic, hydrophobic, or mixed), it is possible to modulate protein adsorption, a key factor in early biological responses, which directly affects cell adhesion, viability, and proliferation.
Materials and Methods: Nitinol surfaces were coated by ALD to create hydrophobic, hydrophilic, and hybrid layers. Coatings were analysed by FIB-TEM-EDS. Wettability was measured by contact angle and surface free energy (SFE). Protein adsorption was assessed quantitatively. Cytotoxicity and fibroblast proliferation were evaluated via direct and extract assays per ISO 10993-5, with proliferation assessed by Alexa Fluor/DAPI staining.
Results: The applied coatings improved Nitinol biocompatibility by reducing cytotoxicity and enhancing protein adsorption. Surface wettability, evaluated by contact angle and SFE measurements, was closely correlated with protein adsorption, indicating that variations in surface hydrophilicity directly influenced the amount and quality of the adsorbed protein layer, which in turn promoted biological integration. Cytotoxicity tests confirmed low toxicity of the coatings. Microscopy analysis supported these findings by revealing favorable cell-material interactions.
Conclusions: ALD coatings with controlled wettability significantly improved Nitinol surface biocompatibility by enhancing protein adsorption and reducing cytotoxicity. The strong correlation between surface wettability and protein adsorption underscores the importance of surface engineering in cardiovascular implants. These findings support the potential of tailored ALD coatings to enhance hemocompatibility and implant integration.
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P.1.37. Polyelectrolyte-Based Systems for Drug Delivery and Cardiac Cell Differentiation

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Background: The development of innovative biomaterials, particularly in the field of functional materials, plays a crucial role in addressing key challenges in regenerative medicine. One of the most promising strategies involves the use of polyelectrolyte-based systems for applications such as targeted drug delivery and tissue regeneration—especially in cardiovascular therapies. This study aimed to design and evaluate multifunctional materials capable of actively supporting biological processes.
Materials and Methods: Polyelectrolyte micelles were formed from natural biopolymers such as chitosan and sodium alginate, designed to function as drug delivery vehicles. The electrostatic interaction between these oppositely charged biopolymers forms stable micellar structures, further enhanced by the incorporation of a thermoresponsive polymer, *N*-isopropylacrylamide (NIPAM). This component enables temperature-sensitive control over polyplex behavior, such as drug release, near physiological conditions (~32°C). A glycogen synthase kinase-3 (GSK-3) inhibitor, a molecule recognized for its ability to stimulate the proliferation of cardiomyocytes, was encapsulated within the micelles. Its incorporation aimed to enhance the regenerative potential of the system by promoting myocardial tissue repair under physiologically relevant conditions.
The cellular response was evaluated using confocal laser scanning microscopy (CLSM), focusing on markers of cell differentiation (GATA-4), proliferation (Ki67), and nuclei (DAPI). The FAK-100 assay kit was employed to analyze cytoskeletal organization, nuclear structure, and the expression of adhesion-related molecules, providing a comprehensive view of the cellular response to the applied materials.
Results: The addition of the drug in micellar form yielded the expected results of increased proliferation and differentiation. Drug-free PEM increased the number of adhering cells.
Conclusions: The developed micellar systems offer a promising multifunctional approach to modulate cellular behavior and promote tissue repair under physiological conditions. These findings support the future application of polyelectrolyte-based materials in regenerative medicine and targeted drug delivery.
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Poster Session 2

Clinical research

P.2.1. Interpretable Machine Learning with SHAP for Glioma Grading Using HLA-DR–Stained Histology and Multi-Feature Analysis

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Background: Gliomas exhibit diverse histopathological features that correlate with tumor grade and immune activity. Immune cell infiltration, especially by myeloid cells expressing HLA-DR, influences prognosis but remains understudied in computational histopathology. Manual grading of such slides is time-consuming and subjective. We propose an interpretable machine learning (ML) pipeline for glioma grade classification (WHO G1–G4) from HLA-stained whole slide images (WSIs), using 4 categories of features - morphological, complexity-based, radiomic, and deep features - with transparent predictions via SHAP explanations.

Materials and Methods: The dataset includes 110 HLA-DR/DP/DQ-stained glioma WSIs. Tiles (1024×1024 px) were extracted with 10% overlap and tissue filtering. Features were obtained from: (1) Cellpose-derived morphology (e.g., area, circularity, solidity, eccentricity), (2) image complexity (IC) metrics (e.g., entropy, fractal dimension), (3) radiomics, and (4) ResNet-50 deep embeddings. K-means clustering (k=2) on shape features categorized cells into "ramified" and "amoeboid" morphological types. We hypothesize that higher-grade tumors exhibit increased prevalence of amoeboid-like phenotypes and increased IC. An SVM classifier will be trained on aggregated features with SHAP.

Results: Preliminary results from a data subset (single G1 and G4 tumors) reveal morphological and structural complexity differences. G4 tumor tiles showed a higher proportion of amoeboid-like cells, consistent with known microglial activation trends. A conservative background subtraction method based on HSV thresholding effectively removes non-tissue regions. Principal component analysis (PCA) of IC features from downsampled WSIs of all available G3 and G4 cases demonstrated that gliomas cluster into distinct subgroups based solely on algorithmically derived IC metrics, highlighting intrinsic visual variations between grades of heterogenous gliomas.

Conclusions: Our ongoing work demonstrates the potential of combining handcrafted and deep features to build interpretable ML models for glioma grading. The integration of 4 feature categories captures relevant immune-related patterns, such as microglia activation. Results will include multi-feature SVM classification with SHAP-based interpretation on the full dataset.

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Preclinical research

P.2.2. Gene-corrected hiPSC model reveals partial dystrophin Dp427 preservation and cardiac Dp116 expression in Duchenne muscular dystrophy

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Background: Duchenne muscular dystrophy (DMD) is caused by mutations in the *DMD* gene, leading to dystrophin deficiency and progressive muscle degeneration. We investigated a rare splice-site mutation (c.9975-1G>T) in intron 68, which may affect expression of dystrophin isoforms Dp427 and Dp116, aiming to understand its molecular and functional consequences.
Materials and Methods: Peripheral blood mononuclear cells from a DMD patient were reprogrammed into human induced pluripotent stem cells (hiPSCs). Using CRISPR/Cas9 gene editing, an isogenic control line was generated by repairing the mutation. Both DMD and repaired hiPSCs were differentiated into cardiomyocytes (hiPSC-CMs) and skeletal muscle cells (hiPSC-SMs). RNA sequencing and western blotting were performed to assess dystrophin expression. Confocal microscopy and functional assays evaluated cellular morphology and β-adrenergic responsiveness.
Results: RNA sequencing revealed that the mutation caused skipping of the first six nucleotides of exon 69, resulting in an in-frame deletion of two conserved amino acids (tyrosine and arginine) in the Dp427 isoform. Western blot confirmed truncated Dp427 in DMD cells. Unexpectedly, the Dp116 isoform—previously considered non-cardiac—was detected in hiPSC-CMs for the first time, suggesting ectopic expression. While Dp427 levels were similar in DMD and repaired hiPSC-CMs, dystrophin was markedly reduced in DMD hiPSC-SMs. Confocal imaging showed membrane abnormalities in DMD hiPSC-CMs, absent in control, indicating impaired dystrophin–β-dystroglycan interaction. Functional tests demonstrated altered β-adrenergic signaling in DMD hiPSC-CMs, with increased beating frequency and faster repolarization after isoproterenol stimulation.
Conclusions: This rare splice-site mutation leads to partial dystrophin preservation via non-canonical splicing alongside with ectopic Dp116 expression and tissue-specific differences impacting muscle cell function. The observed membrane defects and altered β-adrenergic response highlight dystrophin's critical role in cardiomyocyte physiology. Our hiPSC-based model enables detailed study of mutation-specific mechanisms and offers a platform for developing targeted therapies in DMD.
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P.2.3. Decoding Animal Behavioral Patterns with Transformers

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Background: Understanding animal behavior is a key challenge in both ethology and neuroscience. Behavioral analysis plays a crucial role in elucidating the relationship between brain function, environmental stimuli, and observable actions. Accurate behavioral quantification is essential not only for basic research but also for applications in pharmacology, psychiatry, and neurology, where behavioral readouts are often used to assess the effects of genetic modifications or drug interventions. Traditional methods often rely on predefined behavioral categories or manual annotations, which are time-consuming and subject to observer bias. As animal behavior is inherently complex and continuous, there is a growing need for automated, scalable approaches that can extract meaningful structure from raw motion data in an unbiased, data-driven manner.
Materials and Methods: This abstract presents a novel approach for discovering semi-discretized animal behavior structures from motion data using Transformer-VQ (Vector Quantized) models. By combining Transformer-based architectures with VQ quantization, we propose an unsupervised framework that segments and clusters complex animal motions into meaningful behavioral patterns.
Results: The model was tested on datasets from multiple animal species, including those exposed to various psychoactive substances. It consistently segmented motion data into discrete behavioral units, capturing meaningful patterns and transitions. Even under altered physiological states, the model identified stable and interpretable structures, distinguishing both conserved behaviors and drug-induced changes. Quantitative evaluations showed improved clustering quality over baseline methods, supporting the model's robustness and cross-condition applicability.
Conclusions: This approach provides a more granular and interpretable representation of behavior and offers valuable insights into the fundamental building blocks of animal motion. It paves the way for future studies into the neural mechanisms of behavior and offers a robust tool for behavioral research in neuroscience and pharmacology.
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P.2.4. Loss of Nrf2 transcriptional activity affects phenotype and function of macrophages

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Background: Atherosclerosis progression involves chronic inflammation and impaired clearance of apoptotic cells (efferocytosis) within plaques. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcriptional regulator of cellular stress responses. It plays a complex role in atherosclerosis, with studies reporting both pro- and anti-atherogenic effects, depending on cell type and disease stage. Nrf2 expression declines with age, which correlates with increased oxidative stress and inflammation—key drivers of atherosclerosis. The phenotype and function of macrophages, critical in plaque development and resolution, can be affected by Nrf2 activity, but the precise mechanisms remain unclear.
Materials and Methods: Bone marrow-derived macrophages(BMDMs) were obtained from mice with a myeloid-specific transcriptional knock-out of Nrf2 (Nrf2LyzMtKO). Control BMDMs were isolated from appropriate littermate controls. Efferocytosis was assessed by incubating BMDMs with apoptotic thymocytes labeled with pHrodo™ Green and analyzed by flow cytometry. Gene expression of efferocytosis receptors was evaluated using RT-qPCR. Human monocyte-derived macrophages (hMDMs) from healthy volunteers were treated with the Nrf2 inhibitor ML385, polarization markers were assessed by flow cytometry at 24-hour intervals between 24 and 96 hours of treatment.
Results: Nrf2LyzMtKO BMDMs exhibited increased efferocytosis compared to controls, reflecting a functional change associated with reduced Nrf2 activity. Expression of specific efferocytosis receptors (Mertk, CD36) was either lower or unchanged in Nrf2-deficient macrophages, suggesting that enhanced efferocytosis may proceed through mechanisms not dependent on these receptors. In hMDMs treated with ML385, a progressive decrease in CD86 and HLA-DR expression was observed, indicating a shift toward an anti-inflammatory macrophage phenotype driven by Nrf2 inhibition.
Conclusions: Reduced myeloid-specific Nrf2 activity enhances macrophage efferocytosis through receptor-independent mechanisms. Additionally, pharmacological inhibition of Nrf2 in hMDMs promotes a shift toward an anti-inflammatory phenotype. These alterations in macrophage phenotype and function due to diminished Nrf2 activity may influence the progression and resolution of atherosclerosis.
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P.2.5. Diffusion MRI tractography biomarkers to stratify and interpret survival in human glioblastomas

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Background: Glioblastoma (GBM) is a deadly tumor influenced by neural activity, and it likely advances by traveling along white matter pathways. Instead of passive growth, GBM seems to extend broad white matter connections. We therefore proposed that the white matter tracts involved with the tumor form the structural root of this complexity and may consequently hold prognostic significance.
Materials and Methods: We created a lesion-tract density index (L-TDI) based on normative neuroimaging templates in two independent GBM datasets (N=367, N=496). The L-TDI measured the mean fiber density within a mask encompassing all tractography streamlines from the normative atlas intersecting the tumor. Analyses incorporated typical covariates and used Kaplan-Meier plots, multivariate Cox and logistic models, dimensionality reduction strategies, and a machine learning algorithm.
Results: Mean L-TDIs reliably separated survival outcomes (p=0.001, p=0.007; two-sided log-rank test). Median survival was longer for low L-TDI patients in both samples (p<0.001; two-sided Mann-Whitney U-test). In addition, L-TDI reflected tumor shape, location, and white matter spread from normative templates, suggesting GBM spatial patterns and survival effects stem from its interaction with white matter structure. Cox regression indicated L-TDI was an independent prognostic marker (HR=1.07, p=0.005; two-sided Wald's t-test, N=617, df=5), while logistic models showed significant prediction of 12-month survival (p<0.001; two-sided Wald's z-test, N=546, df=6). Likelihood ratio testing validated its unique contribution beyond standard factors in both Cox (p<0.01, df=1) and logistic (p<0.001, df=1) frameworks, with findings consistent across cohorts. Logistic prediction of 12-month mortality yielded balanced accuracies of 0.68 and 0.65, with AUCs of 0.74 and 0.73 using cross-cohort validation.
Conclusions: We investigated GBM as a network-based tumor influenced by its dynamic connections with remote brain areas, showing that tract density imaging provides key information about the structure, positioning, outcome, and progression of human GBM.
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P.2.6. Not infallible! Experimental results refute computational modelling predictions of coumestrol as a virulence inhibitor in *Staphylococcus aureus*

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Background: *Staphylococcus aureus* is a Gram-positive bacterium responsible for numerous life-threatening diseases, including skin infections, endocarditis, and sepsis. Due to its rapidly evolving antibiotic resistance, there is an urgent need for alternative therapeutic strategies. Recent *in silico* studies have proposed coumestrol as a potential anti-virulence agent against *S. aureus*, suggesting that it inhibits the ArlRS two-component system by blocking the dimerization of ArlR. This system regulates the expression of various virulence factors involved in clumping, adhesion, leukotoxin production, and more.
Materials and Methods: *S. aureus* strain LAC was cultivated in Tryptic Soy Broth medium. Expression of the mgrA gene was measured using RT-qPCR. The effects of coumestrol on bacterial growth, survival, gene expression, and virulence-associated phenotypes were assessed using a range of functional assays.
Results: Our findings indicate that at high concentrations, coumestrol inhibits the growth of *S. aureus* and exerts bactericidal effects. However, at sub-inhibitory concentrations, coumestrol does not inhibit the ArlRS system; instead, it appears to enhance virulence by increasing bacterial adhesion to fibrinogen. Furthermore, we found no evidence of synergistic effects between coumestrol and commonly used antibiotics.
Conclusions: These results suggest that coumestrol does not inhibit the ArlRS system and therefore cannot be considered an anti-virulence agent against *S. aureus*. This study highlights the limitations of relying solely on computational predictions in drug discovery and underscores the necessity of experimental validation.
Acknowledgements: This study was supported by an OPUS grant from the National Science Centre, Poland, id: 2022/47/B/NZ6/03379.

P.2.7. Subtype-Specific Proteomic Signatures in Motoneurons, DRG, and Ependyma After Spinalization: Insights into Specialization and Recovery

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Background: Motoneurons (MNs) innervating different muscle groups exhibit distinct physiological and molecular features, influencing their vulnerability to spinal cord injury (SCI). Although subtype-specific MN responses to SCI have been reported, direct comparative proteomic data are lacking. To address this, we analyzed molecular differences between MN subtypes under baseline and post-injury conditions, essential for designing targeted motor recovery strategies. To further characterize the MN circuit environment, we profiled dorsal root ganglia (DRG), providing sensory input, and ependymal cells (EC) lining the central canal, supporting spinal cord homeostasis.
Materials and Methods: We profiled MNs innervating the soleus (SOL), a slow-twitch postural muscle, and the tibialis anterior (TA), a fast-twitch phasic muscle. Adult male Wistar rats (n=19) received retrograde tracers into SOL or TA. After thoracic (Th9) spinal cord transection (SCT), rats were sacrificed at 2 (Sp2W, n=5) or 6 weeks (Sp6W, n=5) post-injury; 9 rats served as controls. Labeled MNs, L3-L5 DRG and EC were isolated by laser microdissection (Leica LMD7000). Proteins were extracted, digested, and labeled with tandem mass tags (TMT). Peptides were separated using an Easy-Spray PepMap column on an UltiMate 3000 nano-LC system coupled to a Q Exactive HF-X mass spectrometer. Data were processed in MaxQuant (v1.6.17.0) with identification via the Andromeda search engine against the *Rattus norvegicus* UniProt database.
Results: We identified ~1200 proteins in MNs, 1500 in EC, and 3000- 5000 in DRGs. Under physiological conditions, SOL MNs exhibited higher than TA MNs expression of proteins linked to enhanced cellular homeostasis and sustained activity, including PICALM (autophagy and endocytosis), CST3 (neuroprotection), LNPK (endoplasmic reticulum structure), HAGH (metabolic detoxification), and CKAP4 (cytoskeleton-ER interaction). Post-SCT, MN subtypes showed distinct proteomic remodeling. In SOL MNs, 35 proteins changed at 2 weeks and 15 at 6 weeks, while TA MNs showed fewer changes (11 and 4, respectively). EC changes were more prominent at 2 weeks, whereas DRG sensory neuron proteomes showed greater alterations at 6 weeks.
Conclusions: Our results reveal both baseline and injury-induced differences between MN subtypes, emphasizing the need for precision approaches in SCI therapy.
Acknowledgements: Supported by MINIATURA 2022/06/X/NZ3/01783 (O. G-W.) and OPUS G2789 (M.S.).

P.2.8. Differential Bioenergetic Responses to TDP-43 Depletion Reveal AMPK-Mediated Motor Neuron Hypermetabolism

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Background: TDP-43 cytoplasmic mislocalization and nuclear depletion are early hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). However, the resulting shifts in neuronal and glial energy metabolism remain poorly defined. Paradoxically, systemic metabolic disorders such as diabetes and dyslipidemia are associated with slower disease progression, suggesting a counterintuitive interplay between TDP-43 dysfunction and cellular energetics. We aimed to characterize these cell-specific metabolic effects.
Materials and Methods: TDP-43 expression was silenced by siRNA in NSC-34 motor neuron-like cells, N2A neuroblastoma cells, and BV2 microglia, with knockdown efficiency confirmed by Western blot. Forty-eight hours post-transfection, glycolytic flux (extracellular acidification rate) and mitochondrial respiration (oxygen consumption rate) were measured using a Seahorse XF Analyzer. AMPK activation was assessed by quantifying its phosphorylation status.
Results: In NSC-34 motor neurons, TDP-43 depletion induced a hypermetabolic phenotype characterized by concurrent increases in glycolysis and oxidative phosphorylation. N2A cells exhibited a hypometabolic response, with reduced rates of both pathways. BV2 microglia shifted toward a predominantly glycolytic profile without changes in respiration. Notably, only motor neurons showed increased AMPK phosphorylation, implicating dysregulated energy sensing as a cell-specific vulnerability.
Conclusions: These findings show that TDP-43 loss triggers cell-autonomous bioenergetic reprogramming: motor neurons become hypermetabolic yet energy-stressed via AMPK dysregulation, while non-motor neuronal and glial cells adopt distinct metabolic adjustments. This mismatch may underlie the selective vulnerability of motor neurons in ALS/FTD. Targeting AMPK-mediated pathways—and validating these findings in vivo—may offer new metabolic therapeutic strategies that complement existing neuroprotective approaches.
Acknowledgements: This work was supported by the EU Joint Programme–Neurodegenerative Disease Research (JPND) (TREMENDOS; UMO-2022/04/Y/NZ5/00122).

P.2.9. Silver and gold nanoparticles release from chitosan matrix – wet chemistry and hybrid approach of bionanocomposites preparation

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Background: Nanotechnology is a field of study that nowadays is an inseparable part of medicine, including the production medical devices and implants. The most scientific reports confirmed the beneficial impact of nanoparticles as bactericidal agents, and moreover, some nanoparticles are useful in drug delivery. However, we must pay attention to the unknown long-range effects of nanoparticle behaviour in the human body. The risk of changes depends on the dose and duration of exposure to the nanoparticles. Besides the unknown impact on the human body, nanoparticles are used according to their unique properties and non-resistance of bacteria for their activity. The principle of applying nanoparticles for biomedical applications is the creation of nanocomposites in processes like precipitating or electrodeposition. The methods have the main drawback: the random distribution of nanoparticles in the polymer matrix, therefore, poor predictable delivery of the antibacterial agents.
Materials and Methods: According to that challenge, we propose two approaches of bionanocomposites preparation: 1) deposition of chitosan with AuNPs or AgNPs layer from colloids and 2) the hybrid preparation of chitosan/NPs nano-sandwich based on spin-coating in chitosan layer fabrication and inert gas condensation technique based on magnetron sputtering in supplying AgNPs and AuNPs.
Results: During the experiments we established that wettability and surface energy are suitable to provide well environment for cells growth. Biocompatibility was tested on osteoblasts (MG-63) in cytotoxicity and flow cytometry experiment. Moreover, the proliferation and potential osteoinductive activity of layers were tested with satisfactory results. In order to controllable dosing of nanoparticles release tests were made in a Ringer solution and examined using the ICP-MS method. Additionally, bactericidal tests were carried out on bacteria strains: *S.aureus* and *E.coli*. The nanoparticle delivery could be limited to an adequate concentration, and a potential hazardous impact could be obviated.
Conclusions: In conclusion, the obtained coatings are biocompatible with MG-63 cells, moreover wettability and roughness parameters indicate a favorable environment for cell growth. Additionally, the coatings exhibit antibacterial activity by limiting bacterial growth on the surface.
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P.2.10. Integrating tumor-connected brain regions into a normative connectome improves prognostic modeling in glioblastoma

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Background: Glioblastoma (GBM) is one of the most malignant types of central nervous system tumors. Recent evidence suggests that GBMs interact structurally and functionally with distant brain regions. Hence, we characterized the topological properties of tumor-associated networks and assessed their relevance to survival rates.
Materials and Methods: Using normative tractography and connectivity mapping, we obtained streamlines that intersected tumors, utilizing survival data from two independent cohorts (N = 367, N = 496). We derived graph metrics from binarized networks, followed by principal components (PC) analysis, T-distributed Stochastic Neighbor Embedding (TSNE), and Uniform Manifold Approximation and Projection (UMAP) to create low-dimensional representations. These features were then incorporated into Cox and logistic regression models alongside sex, age at diagnosis, extent of resection (EOR), and methylation (MGMT).
Results: Initial Cox and logistic regression analyses included sex, age, EOR, and MGMT as covariates. With the exception of sex ($p>0.05$), all clinical variables demonstrated significant prognostic value ($p<0.001$ for age, EOR, and MGMT; Wald's tests). When dimensionality-reduced network features (TSNE/UMAP components) were added to the Cox models, they significantly improved survival prediction compared to clinical variables alone (TSNE: $p<0.0001$; UMAP: $p=0.0024$; likelihood ratio tests, df=2). UMAP analysis revealed significant hazard ratios for both embedding dimensions (HR=1.037, $p=0.003$; HR=0.952, $p=0.02$; Wald's tests). For TSNE, only the second component reached significance (HR=0.981, $p=0.002$), while the first showed a trend (HR=1.003, $p=0.051$). Logistic regression confirmed these effects, with both TSNE and UMAP components improving model fit over baseline ($p<0.001$, likelihood ratio tests). Principal components accounted for >85% of variance, with near-significant hazard ratios (PC1: $p=0.053$; PC2: $p=0.08$) and robust contributions to logistic models ($p<0.01$), both surpassing conventional predictors ($p<0.05$, Wald's tests).
Conclusions: Here we investigated a network model of GBM in the context of prognosis. These preliminary results challenge the traditional paradigm and propose a network-level framework that may enhance prognostic accuracy and enable patient-specific risk stratification.
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P.2.11. Astaxanthin attenuates tactile hypersensitivity by modulation of spinal *IL-6* expression in a mouse model of neuropathic pain

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Background: Neuropathic pain significantly impairs daily functioning and quality of life worldwide, while its conventional treatment often yields poor clinical outcomes. Commonly used analgesics are associated with considerable side effects, and most patients experience only partial symptom relief. These limitations highlight the urgent need for novel therapeutic strategies. This study aimed to evaluate whether astaxanthin - a potent carotenoid antioxidant with documented neuroprotective properties - can alleviate hypersensitivity in a mouse model of neuropathic pain. In addition, we investigated its effect on the expression of pronociceptive interleukins implicated in pain signaling.
Materials and Methods: Neuropathic pain was induced in mice via chronic constriction injury (CCI) of the sciatic nerve. On day 7 post-injury, single intraperitoneal doses of astaxanthin were administered to both male and female mice. Tactile hypersensitivity was assessed using the von Frey test. In a separate experiment, animals received repeated twice-daily astaxanthin treatment for 9 consecutive days, after which spinal cord tissue was collected for analysis of mRNA expression levels of pronociceptive interleukins using RT-qPCR.
Results: Our findings demonstrate that a single administration of astaxanthin effectively reduced tactile hypersensitivity in both male and female mice subjected to neuropathic pain. Moreover, repeated treatment also produced beneficial effects. Gene expression analysis showed that while levels of *IL-1β* and *IL-18* are elevated following CCI, repeated twice-daily astaxanthin administration reduced the expression of the pronociceptive *IL-6*, which may underlie the antinociceptive properties of astaxanthin.
Conclusions: The present study extends the knowledge of the effects of astaxanthin on neuropathy, although further studies should be undertaken to fully elucidate the precise mechanisms of actions of this substance. Obtained results emphasise that antioxidants, which display wide spectre of biological activities, might be promising tool for therapy of neuropathic pain.
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P.2.12. The effect of cytochrome P450 2D6 (CYP2D6) overexpression on the enzyme activity and synthesis of dopamine from tyramine in human neuronal cells.

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Background: The brain dopaminergic system is implicated in different neuropsychiatric diseases (schizophrenia, parkinsonism). Dopamine is formed mainly from L-tyrosine by tyrosine hydroxylase and L-amino acid decarboxylase. However, it can also be synthesized from tyramine by cytochrome P450 2D enzymes (CYP2Ds), as indicated *in vitro* using recombinant enzymes and rat brain microsomes, and *in vivo* in the rat brain microdialysis model. Human CYP2D6 is characterized by genetic polymorphism, which is expressed as a loss or a significant increase in enzyme activity. Thus, changes in the CYP2D6 activity in the human brain may affect both the susceptibility to diseases and the effect of CYP2D6-metabolized drugs. In this study, we have investigated the effect of *CYP2D6* overexpression on the hydroxylation of tyramine to dopamine in human neuronal cells.
Materials and Methods: Human neuroblastoma cell line (SH-SY5Y) of wild-type *(CYP2D6-WT)* and with overexpression of *CYP2D6* gene (*CYP2D6-OX*) was used. Kinetic studies of bufuralol 1'-hydroxylation and tyramine hydroxylation were performed, determining the Km and Vmax values. The activity of CYP2D6 was measured as a rate of bufuralol 1'-hydroxylation (HPLC with fluorescence detection). The formation of dopamine from tyramine by CYP2D6 in neuronal cells was measured using HPLC with coulometric detection.
Results: The *CYP2D6-OX* neuronal cell line displayed much higher activity towards 1'-hydroxylation of bufuralol than the *CYP2D6-WT* cell line. Both cell lines showed a basal level of dopamine synthesized *via* a classical pathway from L-tyrosine. Dopamine was synthesized from tyramine by CYP2D6 in both *CYP2D6-WT* and *CYP2D6-OX* cell lines, reaching a much higher level in the transgenic cell line. The obtained kinetic parameters confirmed the enhancing effect of *CYP2D6* overexpression on the enzyme activity and dopamine formation from tyramine.
Conclusions: Human neuronal cell line can synthesize dopamine from tyramine by CYP2D6. The rate of tyramine hydroxylation to dopamine is significantly higher in the transgenic *CYP2D6-OX* cell line. Considering *CYP2D6* gene polymorphism, the amount of dopamine formed by this enzyme and local drug metabolism may differ between individual subjects. And this may affect the predisposition to neuropsychiatric disorders and drug effects.
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P.2.13. The activity-based rodent model of anorexia and intestinal mucosal defence

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Background: Mucins, renin-angiotensin (RAS), and dopaminergic systems are all involved in maintaining gastrointestinal homeostasis, and emerging evidence suggests potential cross-talk between them, particularly in the context of inflammation. Thus, our aim was to characterise the expression of DOPA decarboxylase (DDC), angiotensin converting enzyme 2 (ACE2), and mucins in the small intestine of Wistar rats in the activity-based animal model of anorexia (ABA).
Materials and Methods: Female Wistar rats (n=12), after the period of acclimatisation under controlled conditions, were randomly assigned to either control or ABA group (with unlimited access to a running wheel and restricted feeding schedule). Fresh specimens from duodenum, jejunum, and ileum were rinsed with PBS (pH=7.4), fixed in paraformaldehyde, routinely processed, and embedded in paraffin. Alcian Blue PAS staining (Sigma Aldrich) was used to identify mucins. For immunolabelling, DOPA decarboxylase mouse monoclonal antibody (Invitrogen CL2962) and ACE2 rabbit monoclonal antibody (Invitrogen SN0754), together with appropriate secondary antibodies (Alexa Fluor 488 and 594) were used. Microscopic slides were examined using an Olympus BX43 epifluorescence microscope equipped with DP74 camera. The qualitative analysis was provided in 20 consecutive fields of vision with 600× (immunofluorescence) or 200x magnification (Alcian Blue PAS).
Results: Mucosal secretory profile was significantly altered in the ABA group as compared with the control. Double immunofluorescence revealed significant co-localization of DDC and ACE2 across the intestinal wall. DDC and ACE2 immunoreactivity was abundantly present in the small intestinal mucosa, submucosa, and both enteric plexuses. Higher immunofluorescence of both enzymes was noted in the enterocytes from the ABA group.
Conclusions: Mucins play a central role in gut barrier integrity and are influenced by inflammatory and neuroendocrine signals. Both renin-angiotensin and dopaminergic pathways can affect mucin expression and enterocyte function, especially under stress conditions, such as the ABA model. Thus, in the ABA model, the above mentioned mechanisms can influence on or result from increased gut permeability, neuroinflammation, and may further sustain maladaptive behaviours.
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P.2.14. Neuroprotective effects of histamine H_3 receptor (H_3R) antagonists in cellular models of Parkinson's disease

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Background: The histamine H_3 receptor (H_3R) is widely expressed in the central nervous system, and its main role is to modulate neurotransmitters release. There are reports suggesting the neuroprotective potential of H_3R antagonists, but their utility for Parkinson's disease (PD) is less recognized. Thus, in this study, we tested the effect of H_3R antagonists in cellular models of PD.
Materials and Methods: We studied the effect of four H_3R antagonists: ciproxifan (CPX), JNJ-5207852 (JNJ), clobenpropit (CB) and pitolisant (Pit) at concentration 0.01-10 µM against cell damage induced by oxidative stress inducer (H_2O_2) and dopaminergic neurotoxins (6-OHDA and MPP^+) in human neuroblastoma SH-SY5Y cells under undifferentiated (UN-) and retinoic acid (RA-) differentiated phenotype. Additionally, we measured expression of H_3R in UN- and RA-SH-SY5Y cells using Simple Protein Jess method.
Results: The expression of H_3R was at similar level in UN- and RA-SHSY5Y cells. H_3R antagonists, when given alone up to 10 µM for 24 hours, did not affect the cell viability of UN- and RA-SH-SY5Y cells. In the H_2O_2 model of cell damage in UN-SH-SY5Y cells, the most protective compounds were CPX (0.1-10 µM) and JNJ (0.01-10 µM), whereas in RA-SHSY, these effects were less pronounced and limited only to the highest tested concentration. In the 6-OHDA cytotoxicity model, the most neuroprotective were CPX (1-10 µM) and CB (0.1-10 µM); however, these properties were not observed in RA-SH-SY5Y cells. Although Pit (0.1 µM) was only slightly protective against H_2O_2-evoked cell death in UN-SH-SY5Y cells, this effect was not observed in differentiated cells, and Pit was not beneficial in the attenuation of 6-OHDA-induced cytotoxicity in both cell phenotypes. In the MPP^+ model, all tested H_3R ligands revealed some neuroprotective effects in the cell viability assay, but only in RA-SH-SY5Y cells. Moreover, this protection was associated with inhibition of MPP^+ induced caspase-3 activity.
Conclusions: H_3R antagonists possess neuroprotective potential in cellular models of Parkinson's disease; however, these effects depend on the type of tested compound, cell damage model, and cell differentiation state. Lower protection mediated by H_3R antagonists in H_2O_2 and 6-OHDA models in RA-SH-SY5Y suggests an involvement of common neuroprotective mechanisms by these ligands and retinoic acid.
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P.2.15. Effect of co-treatment with *N*-acetylcysteine and aripiprazole on the neurodevelopment rat model of schizophrenia

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Background: Schizophrenia is a mental illness of adolescence, affecting about 0.5-1% of the world's population. There are three main categories of symptoms of schizophrenia: positive, negative, and cognitive deficits. The symptoms of schizophrenia are well characterized, but the mechanism underlying the pathogenesis of the disease still remains unknown. In addition, the therapy of negative symptoms and cognitive deficits in schizophrenic patients is a serious clinical problem. Some clinical studies have shown that the atypical antipsychotic drug aripiprazole and the antioxidant *N*-acetylcysteine were effective in reducing positive and negative symptoms of schizophrenia in patients.
Materials and Methods: The aim of the present study was to evaluate the influence of repeated co-treatment with aripiprazole and *N*-acetylcysteine on the schizophrenia-like behavior in adult rats. The schizophrenia-like behavior was induced in Sprague-Dawley male pups in the neonatal days (p5-p16) by repeated administration of the glutathione synthesis inhibitor L-butionine-(*S, R*)-sulfoximine (BSO) given together with dopamine reuptake inhibitor GBR 12909. Adult rats were repeatedly co-treated with aripiprazole (0.1 mg/kg) and *N*-acetylcysteine (10 mg/kg) for 21 days, and their effects on schizophrenia-like behavior were assessed (in p90-91) using the social interaction and novel object recognition test.
Results: The present data indicated that the studied drugs at higher doses: aripiprazole (0.3 mg/kg) and *N*-acetylcysteine (30 mg/kg), reversed schizophrenia-like symptoms in tested model. Moreover, repeated co-treatment at ineffective doses, aripiprazple (0.1 mg/kg) with *N*-acetylcysteine (10 mg/kg) also reversed schizophrenia-like behavior in the neurodevelopmental rat model of schizophrenia.
Conclusions: The above results indicated that *N*-acetylcysteine enhances the action of aripiprazole in the used neurodevelopmental rat model of schizophrenia. Adding *N*-acetylcysteine to aripiprazole in the BSO + GBR 12909 model of schizophrenia leads to a lowering of the dose of this antipsychotic, which is crucial for therapy of both negative symptoms and cognitive functions, and indicates the usefulness of this model to study these symptoms.
Acknowledgments: This study was financially supported by statutory funds of the Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.

P.2.16. Pharmacokinetics, systemic toxicity, and acute behavioural effects of phenethylamine derivative 25E-NBOH in rats

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Background: 25E-NBOH is a novel psychoactive substance derived from phenethylamine, originally synthesized in 2010 for emission tomography. It has emerged as an alternative to classical psychedelics such as LSD, acting as a potent serotonin receptor agonist and reportedly inducing strong visual hallucinations.
Materials and Methods: The study investigates the pharmacokinetics, systemic toxicity, and behavioural effects of 25E-NBOH in rats. Concentrations in brain and serum are measured via LC-MS/MS over 24 hours post-subcutaneous administration. Toxicity is assessed following OECD 423 guidelines. Behavioural effects are evaluated using the Open Field Test and Prepulse Inhibition of Acoustic Startle Response.
Results: Preliminary data suggest significant accumulation of 25E-NBOH in brain tissue, notable behavioural alterations, and systemic toxicity potentially leading to serotonin syndrome. Observations are compared with effects of classical and other novel psychedelics (e.g., LSD, 25CN-NBOH).
Conclusions: 25E-NBOH presents potent psychoactive and toxicological effects in animal models. The findings support the inclusion of this compound in databases mapping the pharmacological profiles of psychoactive substances.
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P.2.17. Identification of Brain Protein Biomarkers in Rats Abstinent from Extended Cocaine Self-administration

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Background: Substance use disorders (SUDs) are chronic, relapsing conditions of the central nervous system, characterized by compulsive drug-seeking and consumption. Cocaine addiction, a major global health problem, leads to intense euphoria, resulting in abuse and addiction. The diagnosis of cocaine addiction is based on criteria from the DSM-5, with drug craving and relapse being central components. This study aims to identify brain proteins regulated in rats abstinent from extended cocaine self-administration using advanced proteomic techniques, leveraging recent progress in mass spectrometry (MS) and neuroproteomics.
Materials and Methods: Wistar Han rats (Charles River, Germany) were implanted with silicon catheters into the jugular vein. After recovery, animals were trained to press an "active" lever for cocaine self-administration and a "non-active" lever as a control. A fixed-ratio reinforcement schedule (FR 1-5) was employed, with rats receiving cocaine (1 mg/kg, 0.1 mL) during 1-hour sessions (5 days) and a reduced dose (0.5 mg/kg, 0.1 mL) during 6-hour sessions (10 days). Light and sound cues accompanied each cocaine injection. After the self-administration phase, rats underwent a 30-day withdrawal period before euthanasia for proteomic analysis. To distinguish motivational from pharmacological effects, self-administering rats were paired with controls receiving passive cocaine or saline injections. Proteomic analysis was performed using SP3-based protein sample preparation and Data-Independent Acquisition (DIA) mass spectrometry. Samples from at least six rats per group were analyzed in duplicate.
Results: Proteomic analysis identified up- and down-regulated proteins in rat brain regions related to craving conditions, such as the dorsal striatum and prefrontal cortex of male and female rats in the cocaine long-term abstinence from self-administration. Several proteins were found to be significantly regulated.
Conclusions: This study identifies novel brain protein biomarkers linked to cocaine abstinence, providing new insights into the neurobiological mechanisms underlying addiction relapse. The results provide a foundation for targeted therapeutic approaches to cocaine addiction, supported by a preclinical model that closely reflects human cocaine consumption patterns.
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P.2.18. Novel Dual Antagonists of 5-HT3 and 5-HT6 Receptors with Expected Antipsychotic Activity

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Background: Schizophrenia is a complex and debilitating neuropsychiatric condition marked by positive symptoms (e.g., hallucinations, delusions), negative symptoms (e.g., apathy, anhedonia), and cognitive impairments affecting memory and executive function. Although pharmacological interventions have been available for over six decades, there is still a need for safe and more efficacious agents. In our recent efforts to search for novel strategies for alleviating the symptoms of schizophrenia, we identified FPPQ, a first-in-class dual 5-HT3R/5-HT6R antagonist. FPPQ inhibited phencyclidine (PCP)-induced hyperactivity and demonstrated pro-cognitive effects in the novel object recognition test.
Materials and Methods: Compound PZ-2609 was identified from a 26-membered library designed using scaffold-hopping approach inspired by FPPQ chemotype. Its affinity for 5-HT6 and 5-HT3R as well the selectivity over 5-HT1A, 5-HT2A, 5-HT7 and D2 receptors was determined in radioligand binding studies. The impact on 5-HT6R constitutive activity of PZ-2609 was tested in in SHSY5Y cells using the BRET method. Metabolic stability was evaluated in vitro in rat liver microsomes. The inhibition of hERG channel was evaluated in radioligand binding assays. Pharmacokinetic studies were performed in Wistar rats.
Results: Compound PZ-2609 exhibited high affinity for 5-HT6 (Ki = 9 nM) and 5-HT3 receptors (93% inhibition at 1 µM) with good selectivity over structurally related serotonin receptors. It functioned as a neutral antagonist at 5-HT6R-mediated Gs signaling. PZ-2609 showed favorable metabolic stability in rat liver microsomes (Clint = 10.3 µL/min/mg), lacked activity at the hERG channel and was brain-penetrant.
Conclusions: Collectively, these findings support further in vivo evaluation of PZ-2609 to elucidate its potential antipsychotic activity in rodents.
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P.2.19. Recording Visual Evoked Potentials in Awake Freely Moving Rats

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Background: Visual evoked potentials (VEPs) offer a non-invasive tool for probing visual system integrity, but their use in awake rodents remains limited. Most previous studies relied on anesthesia, which can distort physiological responses. We introduce a novel method for recording VEPs in awake, head-fixed rats and provide a comprehensive characterization of responses to flash, pattern-reversal, and motion-onset stimuli, including their adaptation dynamics.
Materials and Methods: Ten male Long-Evans rats were implanted with 38 epidural electrodes arranged across the skull. Animals were head-fixed atop a freely rotating sphere, allowing unrestricted body movement. Visual stimuli (100 flash, 200 pattern-reversal, 300 motion-onset) were presented with jittered inter-stimulus intervals (~2.5 s). EEG was recorded at 1000 Hz and preprocessed using MNE-Python. Peak components were extracted from occipital channels overlaying visual cortices. Adaptation was assessed for the dominant positive component using a 95% overlapping sliding window (20 trials per step), with low-pass filtering (20 Hz) to isolate slow components. Spearman correlation determined the association between trial order and P1 amplitude/latency, with significance evaluated via permutation testing.
Results: Responses peaked over the occipital cortex, and each stimulus elicited distinct VEP waveforms. Flash stimulation evoked stereotypical high-amplitude responses (N40, P90), pattern-reversal evoked early P45/N60 peaks, and motion-onset stimuli produced N120/P250 complexes. Responses were maximal over the occipital cortex. Adaptation analysis revealed a robust decrease in P1 amplitude and latency over time for flash stimuli ($r = –0.52$ and $–0.85$, $p < 0.001$), moderate changes for motion-onset ($r = 0.25$ and $–0.17$, $p < 0.01$), and minimal effects for pattern-reversal stimulation.
Conclusions: We demonstrate a robust method for recording high-quality VEPs in awake rats without anesthesia, yielding reproducible responses that closely mirror human VEP profiles. The distinct temporal dynamics across stimulus types reflect underlying differences in visual processing pathways and offer translational potential for rodent models of sensory and cognitive function.
Acknowledgements: Funded by Czech Science Foundation (23-07578K), Ministry of Education (260648/SVV/2024), ERDF (CZ.02.01.01/00/22_008/0004643), VVI CZECRIN (LM2023049), Cooperatio Neurosciences, and PSYRES Foundation.

P.2.20. Senescence-associated pathways shape the angiogenic profile of endothelial cells: Implications for atherosclerosis

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Background: Understanding angiogenesis in atherosclerosis is key to developing therapies targeting vessel growth and plaque stability. While essential for repair, angiogenesis becomes dysregulated in atherosclerosis, resulting in fragile, leaky vessels that destabilize plaques. Senescent cells, which accumulate in vascular tissues, may support this pathological process.
Materials and Methods: This study aimed to characterize the proteome of senescent endothelial cells and assess their impact on angiogenesis. Replicative senescence in Human Umbilical Vein Endothelial Cells (HUVEC) was verified using classical biomarkers such as senescence-associated secretory phenotype (SASP), increased expression of cell cycle inhibitors, lowered proliferation markers, and senescence-associated beta galactosidase (SA-βgal) activity. Cells were then analyzed by LC MS/MS using a highly sensitive technology: Vanquish Neo UHPLC System coupled with Orbitrap Astral. Vascular endothelial growth factor-A (VEGF-A)-induced sprouting angiogenesis was evaluated in low-passage control HUVECs (cEC) and high-passage senescent HUVECs (sEC) using a 3D spheroid assay. Additionally, the angiogenic response of cECs exposed to conditioned media (CM) from cEC or sEC was assessed.
Results: Proteomics revealed 1,032 significantly altered proteins in sEC vs. cEC, with 494 upregulated and 538 downregulated. KEGG and GO-term pathway enrichment analysis highlighted pathways linked to cellular senescence, angiogenesis, and atherosclerosis. Angiogenesis-related proteins were both up- and downregulated, suggesting a dual effect of sEC on vascular development.
In the 3D spheroid sprouting assay, sEC formed significantly less and shorter sprouts than cEC, confirming their reduced intrinsic angiogenic capacity. On the other hand, cEC exposed to CM from sEC developed significantly more and longer sprouts compared to exposure to cEC-derived CM, indicating a paracrine stimulation of sprouting angiogenesis by sEC.
Conclusions: Senescent cells play a dual role in angiogenesis. sEC themselves have impaired ability to form new blood vessels. On the other hand, molecules secreted by sEC can promote angiogenesis in non-senescent endothelial cells. This study identifies key senescence-associated pathways that shape the angiogenic profile of endothelial cells with implications for atherosclerosis.
Acknowledgements: This research was funded by the National Science Centre (NCN) under grant number 2021/43/B/NZ5/03336.

P.2.21. A Wearable Cardiorespiratory Monitoring Solution for Non-Clinical Studies: A novel Technology Supporting Reduction and Refinement in In Vivo Research

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Background: Preclinical cardiorespiratory monitoring in small mammals traditionally relies on invasive implanted telemetry or restrictive plethysmography chambers. Implants necessitate surgery, raising ethical concerns and complexity. Plethysmography, though non-invasive, confines animals, altering natural behavior. To bridge this gap and offer a more humane, accurate solution, a new wearable telemetry system has been designed. This technology provides reliable, robust, non-invasive cardiorespiratory monitoring, enabling high-quality physiological data without surgery, restraint, or isolation.
Materials and Methods: This innovative device features a custom-fit jacket with biosensors, miniaturized on-board electronics for Bluetooth transmission, and analysis software. Developed for small mammals like rats (220-700g) and marmosets (400-500g), the system integrates ECG patches, two inductance plethysmography bands for respiration, and a 3D accelerometer. This setup allows simultaneous, high-fidelity access to cardiac, respiratory, and behavioral data in socialized, freely moving animals. Its modular design permits adaptation to other species by modifying the jacket and algorithms, demonstrating broad versatility.
Results: This wearable technology has been tested across various small mammal species and diverse scenarios, including homecage monitoring, pharmacological studies, pathophysiological investigations, and treadmill exercise. It consistently delivers robust, qualitative signals and relevant physiological data, even in active animals. These studies confirm its ability to accurately capture cardiorespiratory parameters and behavioral insights in real-world, non-stressed conditions. Practical implementation has proven straightforward and efficient across numerous scientific use cases, showcasing its advancements over less reliable external monitoring attempts.
Conclusion: This wearable telemetry represents a major leap in non-invasive cardiorespiratory monitoring for small mammals. It uniquely avoids surgery and confinement, providing accurate, stress-free data comparable to implanted telemetry and plethysmography. By enabling natural behavior and supporting the 3Rs, this robust solution sets a new standard for preclinical research.

P.2.22. Application of Artificial Intelligence in Data Analysis of Nanocomposite Dialysis Membranes

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Background: Chronic renal failure is frequently associated with uremic encephalopathy caused by the accumulation of uremic toxins damaging nerve cells (especially increased concentration of urea, indoxyl sulfate, guanidine compounds, and indolic acid). Clinically, it manifests as cognitive disorders, alterations in consciousness, and seizures. Although it is at least partially treatable with the renal replacement therapy (RRT), current membranes for dialysis are not sufficient enough in clearing Middle Molecular Weight Toxins, which causes the need to further improve its parameters. With the introduction of AI to many areas of science, it might be beneficial to use an AI model for selecting the optimal composition of nanocomposite to provide more effective therapy. The aim of the study was to compare experimental results with algorithm predictions to analyze the relationship between physicochemical parameters and membrane performance.
Materials and Methods: The study used Artificial Intelligence (AI) tools, including the ChatGPT (OpenAI) language model, for the analysis of material data of the series of polysulfone (PSU) dialysis membranes modified with various nanoadditives (graphene, graphite, carbon nanotubes (CNT), silica and core–shell nanoparticles). Predictions were compared with experimental results to assess its accuracy. Experimental results included membrane morphology determined by scanning electron microscopy (SEM), porosity, wettability, surface free energy, and surface Zeta potential.
Results: Wettability analysis showed improved hydrophilicity in membranes modified with silica and CNT, suggesting better interaction with aqueous uremic solutions. The AI model identified core–shell silica as the best-performing nanofiller in terms of predicted neurotoxin removal potential. In addition, the AI model determined porosity and surface wettability as parameters of particular importance for improving the efficiency treatment of uremic encephalopathy.
Conclusions: Since the experimental results showed a non-linear relationship with the composition of tested membranes, the analysis using the AI facilitates design of materials with improved neuroprotective potential. Widely available AI models are good tools for preliminary research, but the differences in predictions and experimental results suggest caution when applying obtained data.
Acknowledgements: This work was supported by “Initiative of Excellence – Research University” (IDUB) grant ID 9735.

Other topic

P.2.23. Evaluation of the functional properties of carbon-carbon composites as potential electrode materials for Deep Brain Stimulation

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Background: Deep brain stimulation (DBS) is an established treatment for movement disorders, particularly Parkinson's disease. Intracranial electrodes used in DBS enable targeted neurostimulation. Currently, electrodes used in DBS are typically made from metals such as platinum, which are biocompatible but susceptible to corrosion over time. It influences their safety and long-term efficiency. Moreover, the stiffness and high impedance of metal electrodes limit their potential for miniaturization. These challenges highlight the need to develop alternative electrode materials.
Materials and Methods: In this study carbon-carbon composites consisting of carbon fibers in a pyrolytic carbon matrix surface modified with highly oxidised carbon nanotubes and without surface modification, were examined to evaluate their suitability for use in DBS. Hydrogels composed of sodium alginate and gelatin were used to simulate brain tissue. The electrode materials were characterized using digital microscopy and scanning electron microscopy (SEM). The wettability of the carbon composites was assessed, and mechanical tests simulating conditions during DBS surgery were conducted.
Results: The mechanical testing methodology was optimized to account for the high slenderness and small dimensions of the electrodes. SEM analysis following mechanical simulations revealed no significant structural damage that would influence electrodes' integrity, even under forces exceeding those typically occurring during DBS implantation surgery.
Conclusions: Our work provides insight into the mechanical performance of carbon composite micro-electrodes. The results showed that the analyzed electrodes are promising candidates for replacing metallic materials in neural stimulation in terms of their mechanical properties.
Acknowledgements: This research was funded in whole by the National Science Centre, Poland, grant number: UMO-2020/39/B/ST5/02126. Hybrid carbon composites for stimulation of cells of the central nervous system.

P.2.24. The cytotoxic effects of novel third-generation antipsychotic drugs on 3D HepaRG spheroids.

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Background: Antipsychotic drugs are used to treat schizophrenia and other psychotic-related symptoms. They are administered to patients for a long time, often in combination with other drugs that are substrates of cytochrome P450 (CYP) enzymes. Brexpiprazole and lumateperone are new atypical antipsychotic drugs, which were approved for the treatment of schizophrenia and depression. Spheroids, which are cellular 3D aggregates, constitute a good model for studying long-term effects of drugs on hepatocyte biochemistry and functioning, and these properties render this model suitable for supporting chronic toxicity tests.
Materials and Methods: Experiments were carried out using HepaRG spheroid culture. After spheroid formation (day 5), the cultures were treated with different concentrations of brexpiprazole or lumateperone (0.05-200 µM). The cell viability (ATP concentration) was measured on days 7, 14, 21, 28, and 35 of culture.
Results: Brexpiprazole was cytotoxic for spheroids only in the highest concentration (200 µM), even in 35 days of culture. Lumateperone was cytotoxic for HepaRG spheroids in the high concentrations examined (200, 100, 50 µM). The cytotoxic concentrations of both examined drugs significantly exceeded therapeutic levels observed in the blood serum of patients.
Conclusions: Brexpiprazole and lumateperone are not toxic to human hepatocytes after long-term treatment with therapeutic concentrations. Further studies are being conducted to test the possible effects of brexpiprazole and lumateperone on liver function and drug-metabolizing enzymes.
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P.2.25. Enhancing Photodynamic Applications of Carbon Dots through Nitrogen and Sulfur Doping

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Carbon dots are modern nanomaterials that are gaining increasing interest in the high-tech field due to their optical properties, high stability, and non-toxicity. Their easy, inexpensive, and environmentally friendly method of synthesis opens up a wide range of applications, especially in areas such as biomedicine, photocatalysis, and environmental protection. At the same time, despite a growing number of studies, little is still known about the effects of chemical composition and the presence of heteroatoms on the mechanisms of action of these materials.

The present study analysed a series of carbon dots modified with nitrogen and sulphur, focusing on two basic processes: energy transfer and electron transfer, which are responsible for the formation of various reactive forms. The results showed that the appropriate choice of material composition and structure makes it possible to control the dominant reaction mechanism. In particular, nitrogen doping increases the efficiency of singlet oxygen generation, while the appropriate configuration of sulphur in the dot structure promotes electron transfer and hydroxyl radical production.

Understanding these relationships opens up new opportunities to design functional nanomaterials tailored to specific applications, especially in biomedicine. Carbon dots can be used in photodynamic therapy and the controlled generation of radicals necessary for effective light-based therapies, paving the way for innovative treatment methods.

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P.2.26. Development of a Selective Allosteric Activator Targeting the PERK Pathway

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Background: Diabetes and cancer are non-communicable diseases with increasing prevalence worldwide and have been associated with oxidative stress, inflammation, and endoplasmic reticulum (ER) stress. Under conditions of ER stress, the Unfolded Protein Response (UPR) is mediated by three signaling transmembrane proteins—IRE1, ATF6, and PERK. While PERK hyperactivation is linked to these conditions, genetic silencing of PERK has instead been shown to result in glucose dysregulation, suggesting that a basal level of PERK activity is essential for cellular function.
Materials and Methods: PERK crystal structures were downloaded from the Protein Data Bank, including both the DFG-in and DFG-out conformations, to evaluate the binding affinity and interaction with the activator MK-28 at the allosteric site of the kinase, using ligand docking tool and molecular dynamics (MD) simulations, in the Maestro Schrödinger software package, to predict the stability of the ligands. The AI-based *de novo* drug discovery tool iGen, in the i-TripleD AI/ML-based platform, was used to identify potential binders of PERK and design new chemical compounds.
Results: The ATP-binding site and allosteric site were mapped using the structures of the substrate ATP and the activator MK-28, respectively. Docking studies showed that the ATP-binding affinity scores increased after MK-28 was bound to the allosteric site. MD simulations were performed using both the active and inactive kinase complexes with MK-28, and displayed higher stability in the DFG-out conformation. Based on the structures of MK-28, a new ligand named 28b was designed in accordance with Lipinski's Rule of Five. MD simulations in triplicate showed that 28b demonstrated increased stability in the allosteric site compared to MK-28. Finally, three analogs of 28b were synthetized for upcoming *in vitro* testing.
Conclusions: In general, the results of this project will provide a broader understanding of the PERK activation effects and establish a basis for selective and safe treatment of cell viability recovery in diabetes, as well as new therapeutic routes for several cancers and metabolic diseases.
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P.2.27. High-calcium bioactive borate glasses for tissue engineering applications

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Background: Bioactive borate glasses (BBGs) are considered promising materials for regenerative medicine due to their ability to deliver biologically active boron species. Boron plays a key role in metabolic processes, supports wound healing, and improves bone health. The incorporation of boron into glass matrices significantly influences the structure, processing, degradation, and biological response of the materials. Due to their lower network connectivity compared to silicate glasses, BBGs degrade faster and can fully convert to hydroxycarbonate apatite (HCA).
Materials and Methods: High-calcium borate glasses with a molar composition of 54CaO-40B_2O_3-6P_2O_5 (A2B40) were synthesized using both melt-quenching and sol-gel methods. Structural characterization was carried out using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Magic Angle Spinning Nuclear Magnetic Resonance (MAS NMR). A simulated body fluid (SBF) assay was conducted to evaluate *in vitro* bioactivity. The surface transformation of the glasses was analyzed using FTIR and Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM/EDS). Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was employed to investigate the release kinetics of B ions and changes in Ca and P concentrations in the incubation solution.
Results: XRD confirmed the amorphous structure of the synthesized glasses, with minor crystallization observed in the melt-derived sample. FTIR and NMR analyses revealed the presence of both trigonal and tetrahedral borate units. After incubation in SBF, FTIR and SEM/EDS analyses confirmed the transformation of the glass surface into an apatite-like layer.
Conclusions: High-calcium borate glasses obtained by melt-quenching and sol-gel methods exhibited favorable structural characteristics and underwent surface transformation into an apatite-like layer *in vitro*. Their dissolution behavior and ion release profile make them promising candidates for applications in tissue engineering.
Acknowledgements: This work was supported by the „Excellence Initiative – Research University" program for AGH University of Krakow.

P.2.28. Towards transparent corneal substitutes: bioprinted models with tuned geometry and light transmission

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Background: Corneal blindness is a major global cause of vision loss, largely due to limited donor tissue. To address this, research focuses on artificial corneal implants with high optical transparency, up to 90% in the human cornea. Bioinks for 3D bioprinting have shown transmittance from 71.9% to 94%, but scalable, reproducible methods that ensure both optical clarity and mechanical and biological compatibility are still needed. Despite promising results, there is still a need for scalable and reproducible printing methods that offer optical, mechanical, and biological compatibility.
Materials and Methods: In this study, a 3D bioprinting approach using extrusion in a Pluronic-based support bath containing calcium chloride was employed to fabricate corneal implant models. The bioink formulation was based on sodium alginate, optimized for printability and crosslinking. Two types of samples were fabricated to analyze geometry-dependent optical behavior: flat, disc-shaped constructs and dome-shaped models mimicking corneal curvature. Before testing, samples were hydrated in distilled water to simulate physiological conditions. Transmittance measurements were conducted using a UV-2600i spectrophotometer (Shimadzu) across the 400–800 nm wavelength range with 1 nm resolution.
Results: The spectrophotometric analysis revealed geometry-dependent transmittance: flat disc-shaped samples demonstrated light transmittance of approximately 80%, dome-shaped corneal models reached up to 88% across the visible spectrum. These results are comparable to values reported in the literature and fall within the target range for optical corneal substitutes. Moreover, the constructs exhibited favorable structural integrity post-printing, with dome-shaped models maintaining curvature without collapse. Mechanical stability and optical clarity were preserved throughout the 12-day ex vivo incubation period.
Conclusions: The presented method demonstrates that extrusion-based 3D bioprinting in a Pluronic support bath can yield corneal models with clinically relevant optical properties. The achieved light transmittance, particularly in dome-shaped structures, meets the threshold necessary for potential application as artificial corneal implants. This work supports the feasibility of using sodium alginate-based bioinks in fabricating geometrically accurate, transparent constructs, paving the way for future investigations into functionalized versions for regenerative ophthalmology.

P.2.29. Polymers in the Service of Neuroscience

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Background: Among the diverse methodologies currently available, nerve tissue engineering (NTE) has emerged as a promising approach for the reconstruction of damaged nerve tissues. A critical factor determining the efficacy of such medical interventions is the choice of material employed.
Materials and Methods: Conducting polymers have garnered considerable interest in tissue engineering applications owing to their intrinsic capacity for charge transport. Their electrical activity and reversible doping processes enable the transmission of both electrical and mechanical stimuli. Furthermore, these polymers provide a biocompatible scaffold that offers physical support for living cells. Additionally, mechanical properties such as scratch resistance and adhesion are examined, as these characteristics are crucial for prospective coating applications.
Results: Practical applications of conducting polymers in neuroscience include their use in the fabrication of neural electrodes capable of recording and stimulating neural activity with improved signal fidelity and reduced inflammatory response. For instance, polypyrrole-coated electrodes have demonstrated enhanced electrical conductivity and biocompatibility, contributing to better long-term performance in neural implants. Moreover, conducting polymer-based scaffolds are being developed to guide axonal growth in peripheral nerve regeneration, offering an alternative to traditional nerve grafts.
Conclusions: The CP-based scaffolds can be engineered to release growth factors or drugs in response to electrical stimulation, thereby promoting nerve healing in a controlled manner. These examples underscore the significant potential of conducting polymers as multifunctional materials, bridging the gap between electronic devices and biological tissues in the field of neuroscience.
Acknowledgements: The work was supported by the Institute of Materials Engineering's own funds, which provided the resources necessary to conduct the research.

P.2.30. Bioinformatics in hospital: bone marrow transplant in Thalasemia patients in Leiden University Medical Center.

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Background: Thalassemia is a genetic blood disorder characterized by impaired hemoglobin synthesis, resulting from mutations that disrupt the structure of this protein. Currently, there is no effective pharmacotherapy: life-long management relies on regular blood transfusions and iron chelation therapy. Unfortunately, these procedures are associated with severe complications—including iron overload, organ damage, and reduced overall survival. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only potentially curative option, yet its success in thalassemia patients is limited by high rates of graft rejection and graft-versus-host disease (GvHD).
Materials and Methods: We performed single-cell RNA sequencing (scRNA-seq) on bone marrow samples obtained from healthy donors and thalassemia patients before and post-transplantation. Data were processed and analyzed using bioinformatics tools (such as Seurat, SingleCellExperiment), and cell populations were visualized via CellxGene.
Results: We were able to accurately identify the majority of the cell's origin—whether from the patient or the donor in post-transplant samples. Preliminary analysis revealed differences between patient and donor cells, particularly in the final stages of erythropoiesis. These findings suggest that, in the context of transplantation, signals from the patient's bone marrow environment—or residual host cells—may affect donor cell behavior, potentially leading to altered function and contributing to graft failure.
Conclusions: Our data suggest that the interaction between donor cells and the thalassemic bone marrow niche plays a key role in transplant outcomes. Incorporating the identified genetic and antigenic markers into donor–recipient matching protocols may help reduce rejection rates and improve the success of hematopoietic stem cell transplantation (HSCT) in thalassemia patients. Ongoing research aims to validate these candidate markers in larger cohorts and to explore strategies for modifying the host microenvironment before transplantation.

P.2.31. Bioactive borate glasses in a B_2O_3-CaO system for hard and soft tissue regeneration

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Background: Bioactive glasses are biocompatible and support the formation of new bone tissue, contributing to the regeneration process. The most commonly used bioactive glasses are silicate-based, primarily applied in bone tissue regeneration. However, due to their slow degradation rate and incomplete dissolution, they are less suitable for applications in soft tissue regeneration. As an alternative, materials with faster degradation rates, such as phosphate or borate glasses, are being developed. Bioactive borate glasses (BBG) are a group of materials that can be used in chronic wound treatment, provide controlled ion release, and serve as biodegradable scaffolds for cells. BBGs convert to hydroxyapatite (HAp) significantly faster than silicate glasses, which offers them an advantage in medical applications. This faster dissolution is attributed to the lower chemical durability of borate glasses. The ion release from bioactive glasses can also be utilized in soft tissue applications by stimulating natural healing processes.
Materials and Methods: This study focuses on synthesizing and characterizing BBGs to assess how different synthesis methods impact their bioactivity and structural properties. The glasses were developed in a binary oxide system ($80B_2O_3$-20CaO mol%) using two approaches: traditional melting and the sol-gel technique. Techniques used for structural and phase analysis as well as bioactivity evaluation of the biomaterials, included FTIR, XRD, NMR, SEM, and ICP-OES.
Results: Glasses exhibit semi-crystalline structure, while FTIR analysis revealed the presence of borate in both tetrahedral and triangular coordination; however, the relative ratio of these units depends on synthesis methods. To evaluate the bioactivity, the BBGs were immersed in SBF, and analysed for apatite formation—a key marker for bioactive potential. After SBF immersion, FTIR, SEM, and ICP-OES analyses verified BBGs transformation into calcium-phosphate phase, indicating their bioactivity and compatibility with bone-like tissue.
Conclusions: The produced bioactive borate glasses can serve as highly functional therapeutic materials in powder form, specifically designed to support the regeneration of soft and hard tissues.
Acknowledgements: This work was supported by the “Excellence Initiative – Research University” program at AGH University.

P.2.32. Synthesis of new phenylcoumarins derivatives for fluorescent sensors

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Background: Fluorescent sensors are analytical tools that utilize fluorescence to detect or quantify specific chemical, biological, or physicochemical parameters in the environment, such as pH, presence of heavy metals, or toxins. Such sensors use fluorophores, whose light-emission properties change depending on the presence of the analyte. Changes may involve, e.g., different fluorescence intensity, altered emission wavelength, or fluorescence lifetime.
Materials and Methods: The aim of the presented work focuses on the synthesis and potential utility of new phenylcoumarin derivatives obtained via Buchwald reactions. Crude products were purified using column chromatography and crystallization. Subsequently, purified products were studied for their light-emitting properties. Absorption spectra, photolysis tests, and fluorescence measurements were performed to describe spectroscopic properties of the presented compounds.
Results: A series of new phenylcoumarin compounds was designed and synthesized, incorporating different withdrawing substituents to optimize their photophysical properties, such as emission wavelength and fluorescence quantum yield. The newly obtained derivatives exhibited a bathochromic shift in the absorption spectrum compared to commercial analogs.
Conclusions: The results indicate that properly designed phenylcoumarin derivatives can serve as promising platforms for the development of modern fluorescent sensors with high sensitivity and selectivity. The structural versatility of phenylcoumarins allows for targeted functionalization, enabling fine-tuning of their fluorescence behaviour to suit specific analytical needs. These findings support the further exploration of phenylcoumarin-based systems as promising candidates for advanced sensing applications in both research and applied contexts.
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P.2.33. Development and characterization of carbon-ceramic nanofibrous membranes CNFs-SiC for water purification applications

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Background: The global water crisis, driven by industrialization, urbanization, climate change, and environmental degradation, demands effective purification technologies. Membrane filtration is commonly used but suffers from fouling, especially biofouling, reducing both efficiency and membrane lifespan. Carbon nanomaterials like nanotubes and graphene have shown potential, but carbon nanofibers (CNFs), though less studied, offer similar advantages. This study focuses on the development of carbon-ceramic nanofibrous membranes by modifying CNFs with a ceramic phase introduced via silicon carbide (SiC) precursors to enhance membrane performance and durability.
Materials and Methods: Electrospinning was used to fabricate CNFs with controlled morphology and composition. Polysiloxane, as a SiC precursor, was added to the spinning solution. The fibers underwent a multi-step thermal treatment process: stabilization, carbonization, and high-temperature processing. The resulting carbon-ceramic composites combined the beneficial properties of both phases. SiC nanostructures enhance mechanical strength, chemical and thermal resistance, and oxidation stability. The materials were characterized using SEM, XRD, FTIR spectroscopy, and surface zeta potential analysis. Antibacterial tests were performed to assess activity against *Staphylococcus aureus* and *Escherichia coli*.
Results: SEM revealed SiC nanostructures on the CNF surfaces. XRD confirmed the presence of the β-SiC polytype. The antibacterial tests showed a certain reduction in both Gram-positive and Gram-negative bacteria, likely due to mechanical damage caused by needle-like SiC structures and the generation of reactive oxygen species.
Conclusions: Carbon nanofibers modified with β-SiC nanostructures were successfully developed. The resulting nanofibrous membranes are hydrophobic and exhibit antibacterial properties. The presented method shows promise for producing durable, antifouling materials suitable for water purification applications.
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P.2.34. Modelling Glycosylated Neurotransmitter Receptors reveals Glycan-mediated Constraints on Ion Conduction

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Background: Neurotransmitter receptors are fundamental to synaptic communication, yet their function is often computationally studied in simplified models that neglect post-translational modifications like glycosylation. The functional role of native glycans is often ignored in pharmacological research as a result, leading to a potentially flawed understanding of signalling mechanisms and inaccurate conclusions about receptor pharmacology. Here, we provide a comprehensive pipeline for glycan-aware computational modelling of neurotransmitter receptors, with an initial focus on the clinically significant $GABA_A$ receptor.
Materials and Methods: A computational pipeline integrating homology modelling, our in-house tool for glycan shielding prediction: GlycoSHIELD, and molecular dynamics simulations was developed to construct a glycosylated model of the α1β3γ2 subtype $GABA_A$ receptor. Using microsecond-scale molecular dynamics simulations, we examined how glycans affect ion entry in the receptor vestibule, with particular focus on glycoforms linked to neurological diseases.
Results: In our simulations, we observe N-glycans extending into the vestibule (inner glycans) constrict the ion conduction pathway, affecting the mean first passage time for the chloride ion to enter, and the ability of glycans to block ionic current seems to depend on their conformation, with native glycan position seen in cryoEM being most permissive. We also observed changes in protein surface shielding by glycans near ligand and drug binding sites, which may directly affect drug affinity, aligning with research on modified glycans and neurological disorders.
Conclusions: Our findings emphasise that overlooking glycosylation leads to an incomplete and potentially incorrect view of neurotransmitter receptor pharmacology. A systematic examination of additional clinical targets, including glycine, AMPA, and NMDA receptors, is currently in progress to build an accurate understanding of synaptic signalling.
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P.2.35. ITS1 and 16S metabarcoding of soil fungal and bacterial genomic DNA samples – a new tool in forensic investigation

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Background: Soil fungal and bacterial communities may serve as region-specific microbial signatures. In forensic contexts, the detection and comparison of such signatures can support investigations by linking suspects to crime scenes.
Materials and Methods: The project focused on defining a fungal- and bacterial-based soil signature for the Katowice district using ITS1 and 16S metabarcoding. A total of 60 soil samples were collected across four seasons from five representative zones within the district, with three samples obtained per site. Metagenomic DNA was extracted, and ITS1 and 16S amplicon libraries (n=60) were prepared. Sequencing was performed on the AVITI platform using 2 × 300 nt paired-end reads, with a minimum of 50,000 read pairs per library generated via avidity-based technology. Bioinformatic analysis was conducted using the QIIME pipeline and the UNITE reference database. Processing steps included read filtering, clustering into ASVs, species-level identification, and diversity analysis.
Results: Phylogenetic trees and similarity plots were constructed, and comparative analysis between sampling groups was undertaken. Relationships between the fungal and bacterial community structure and concentrations of heavy metals and petroleum-derived compounds were assessed. This biological and chemical data integration was particularly relevant in an industrialised urban setting such as Katowice, yielding robust and meaningful correlations.
Conclusions: This study constitutes the first attempt to characterise a regional forensic soil trace in the capital of the Silesian Voivodeship, Poland, based on fungal and bacterial DNA.
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P.2.36. The role of 5-HT2A receptors in sleep architecture and memory consolidation in the animal model

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Background: This study investigates the role of the serotonergic system, specifically the 5-HT2A receptor, in sleep architecture and memory consolidation in an animal model. Psilocybin, a neuroplastic serotonergic psychedelic, is currently explored for its therapeutic potential in psychiatric disorders.
Materials and Methods: Animals (15) were implanted with EEG/EMG telemetry devices to enable continuous sleep monitoring. Treatments were administered subcutaneously, and the animals were then either left undisturbed or underwent 8 hours of gentle handling for sleep deprivation during the light phase. To evaluate memory consolidation, animals performed the object-place recognition task and the Morris water maze. Memory testing occurred following the sleep recording period to assess delayed effects.
Results: Psilocybin and MDL100907 did not significantly affect NREM sleep but produced marked alterations in REM sleep. REM onset latency increased significantly in all drug-treated groups compared to controls, and REM duration was significantly reduced. Psilocybin also increased wakefulness in the light phase post-injection, suggesting a shift in arousal regulation. Sleep deprivation itself reduced both NREM and REM sleep, confirming the effectiveness of the protocol. However, no significant effects were found on memory performance in either the OPR task or the MWM probe test.
Conclusions: In conclusion, while sleep deprivation reliably impaired sleep architecture, it did not significantly disrupt memory consolidation in the employed paradigms. Acute 5-HT2A receptor modulation selectively disrupted REM sleep architecture without affecting NREM sleep or behavioural measures of memory consolidation. The observed changes in REM suggest that serotonergic systems may regulate sleep phases independently of cognitive outcomes in the short term.
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