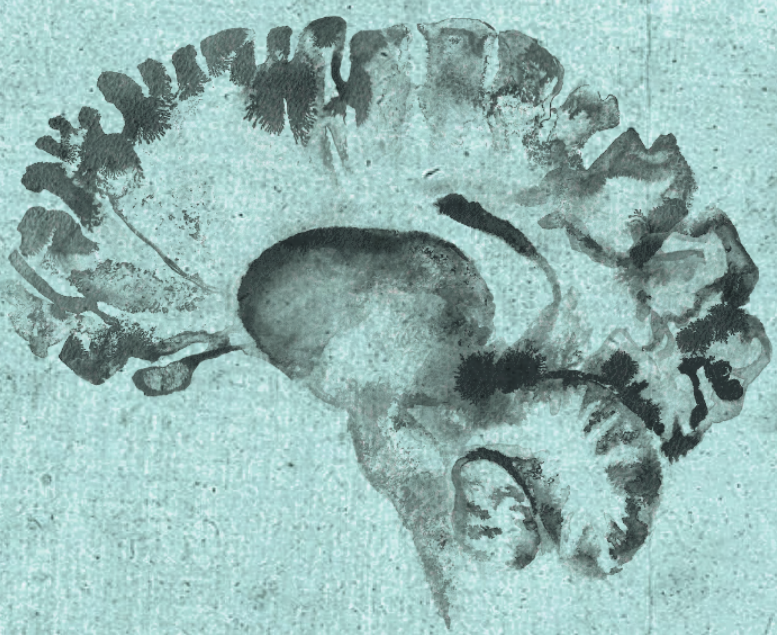
ISSN 0065-1400 (PRINT)
ISSN 1689-0035 (ONLINE)

**2026 Volume 86**
**Supplement**

# ACTA NEUROBIOLOGIÆ EXPERIMENTALIS

www.ane.pl



24-26 APRIL 2026

KRAKÓW

## THE 14TH NEURONUS & YOUNG PTBUN NEUROSCIENCE FORUM

# THE 14TH NEURONUS & YOUNG PTBUN NEUROSCIENCE FORUM

24th to 26th of April 2026, Jagiellonian University, Cracow, Poland

## CONTENTS

# ORGANIZERS

**STUDENT NEUROSCIENCE SOCIETY 'NEURONUS'**

Institute of Zoology and Biomedical Research, Jagiellonian University

**YOUNG PTBUN**

Junior Committee of the Polish Neuroscience Society

**NEUROBIOLOGY COMMITTEE OF THE POLISH ACADEMY OF SCIENCES**

## Head of the Conference

Adrianna Zeńczak
*Jagiellonian University, Cracow*

Martyna Bernaciak
*Jagiellonian University, Cracow*

## Academic Organizers

Coordinator – Dr Michał Ślęzak
*Lukasiewicz Research Network – PORT, Wroclaw*

Coordinator – Dr hab. Michał Kuniecki, prof. UJ
*Jagiellonian University, Cracow*

Dr Aleksandra Trenk
*Jagiellonian University, Cracow*

Dr Ilona Kotlewska
*Jagiellonian University, Cracow*

Dr Aleksandra Domagalik-Pittner
*Jagiellonian University, Cracow*

Dr Paweł Boguszewski
*Nencki Institute of Experimental Biology PAS, Warsaw*

## Members of Organizing Committee

Khuslenzaya Hunter
*Jagiellonian University, Cracow*

Magdalena Chodlewska
*Jagiellonian University, Cracow*

Magdalena Siwarga
*Jagiellonian University, Cracow*

Emil Wawak
*Jagiellonian University, Cracow*

Dominika Hury
*Jagiellonian University, Cracow*

Pola Szerszon
*Jagiellonian University, Cracow*

Katarzyna Deplewska
*Jagiellonian University, Cracow*

Artur Wójcik
*Jagiellonian University, Cracow*

Jakub Jurzec
*Jagiellonian University, Cracow*

Julia Tesarczyk
*Jagiellonian University, Cracow*

Anna Smolarska
*Jagiellonian University, Cracow*

Natalia Kurek
*Jagiellonian University, Cracow*

Julia Stawiarz
*Jagiellonian University, Cracow*

Maria Kowalska
*Jagiellonian University, Cracow*

Mateusz Zaborski
*Jagiellonian University, Cracow*

Emil Dzierkacz
*Jagiellonian University, Cracow*

Karolina Broniszewska
*Jagiellonian University, Cracow*

Natalia Gaik
*Jagiellonian University, Cracow*

Kinga Kruszewska
*Jagiellonian University, Cracow*

Dominika Moskal
*Jagiellonian University, Cracow*

Aleksandra Bartnik
*Aalto University School of Science, Espoo, Finland*

Tomasz Walkiewicz
*Aalto University School of Science, Espoo, Finland*
*& University of Helsinki, Helsinki, Finland*

Katarzyna Wiktor
*Jagiellonian University, Cracow*

## SCIENTIFIC COMMITTEE

### Scientific Committee Biological Section

Dr Michał Ślęzak
*Lukasiewicz Research Network – PORT, Wrocław*

Dr hab. Anna Błasiak, prof. UJ
*Jagiellonian University, Cracow*

Dr Ali Jawaid
*Lukasiewicz Research Network – PORT, Wrocław*

Dr Aleksandra Trenk
*Jagiellonian University, Cracow*

Dr Steffen Kandler
*FMI, Basel*

Prof. Gilles van Luijtelaar
*Radboud University, Nijmegen*

Dr hab. Piotr Majka
*Nencki Institute of Experimental Biology PAS, Warsaw*

Dr Klaudia Kwiatkowski
*The University Hospital in Cracow*

Dr Agnieszka Krzyżosiak
*Lukasiewicz Research Network – PORT, Wrocław*

### Scientific Committee Cognitive Section

Dr hab. Michał Kuniecki, prof. UJ
*Jagiellonian University, Cracow*

Dr Aleksandra Domagalik-Pittner
*Jagiellonian University, Cracow*

Dr Ilona Kotlewska
*Jagiellonian University, Cracow*

Dr Przemysław Adamczyk
*Jagiellonian University, Cracow*

Dr hab. Michał Bola, prof. UJ
*Jagiellonian University, Cracow*

Dr hab. Mirosław Wyczesany, prof. UJ
*Jagiellonian University, Cracow*

Dr hab. Jakub Szewczyk
*Jagiellonian University, Cracow*

Dr hab. Marek Binder, prof. UJ
*Jagiellonian University, Cracow*

Dr hab. Szymon Wichary, prof. UJ
*Jagiellonian University, Cracow*

Dr hab. Eligiusz Wronka, prof. UJ
*Jagiellonian University, Cracow*

**Poster design**
Irmina Mendera
irmina.mendera@gmail.com

**Contact**
neuronusforum@uj.edu.pl

## HONORARY PATRONAGE

Prof. Magdalena Chadzińska
*Dean of the Faculty of Biology of the Jagiellonian University*

Prof. Elżbieta Przybył-Sadowska
*Dean of the Faculty of Philosophy of the Jagiellonian University*

Prof. Michał Wierzchoń
*Head of the Institute of Psychology of the Jagiellonian University,*
*Head of the Centre for Brain Research*

## PARTNERS

The Marceli Nencki Foundation for Supporting Biological Sciences

The Polish National Agency For Academic Exchange (NAWA)

Prof. Krzysztof Pyrć
*President of Foundation for Polish Science*

Fundacja Młodej Nauki

Fulbright Poland

International Brain Research Organization (IBRO)

Program “Open Science” of the Polish Academy of Sciences

Jagiellonian University Foundation for Students and Alumni “Bratniak”

## SPONSORS

## MEDIA PATRONS

Pismo Studentów WUJ

# PROGRAMME

## 23.04, THURSDAY

**10:00-18:00** **WORKSHOPS**

**19:00-21:00** **Science Jam "Career Paths", Hevre – sponsored by IBRO**
*With Michał Bola, Ali Jawaid, Judith Schweimer*
*Moderator: Michał Ślęzak*

## 24.04, FRIDAY

**9:00-10:30** **OPENING CEREMONY**
*Adrianna Zeńczak, Martyna Bernaciak (Heads of Neuronus 2026)*
*Michał Ślęzak (Neuronus Neuroscience Forum Coordinator)*

**Presentation of IBRO activities**
*Daniel Wójcik (chair of IBRO-PERC)*

**KEYNOTE LECTURE: "Conscious Vision and Its Restoration in Blindness"**
*Pieter R. Roelfsema, Netherlands Institute for Neuroscience, Amsterdam & Institut de la Vision, Paris, France*
*Chair: Jakub Szewczyk*

**10:30-11:00** **FLASHTALKS**
*Chair: Magdalena Siwarga, Martyna Bernaciak*

**11:00-11:30** **Coffee break**

**11:30-13:00** **SYMPOSIA SESSION:**

**"High-Level Feedback in Vision: Rules of Prediction, Suppression, and Filling-In"**
*David Richter, Jakub Szewczyk, Mandy Bartsch, Łukasz Bola*
*Chair: Jakub Szewczyk*

**"Touch, Pain and Interoception in Somatosensory and Cancer Neuroscience"**
*Andrew Bell, Mateusz Kucharczyk, Gabriela Basile Carballo, Marek Brodzki, Aleksandra Herman*
*Chair: Felipe Meira de-Faria*

**"Brain Diseases – Different Models, New Insights"**
*Alexander Whitworth, Justyna Kadłuczka, Zuzanna Kula, Chin Long Poo, Aleksandra Klachacz*
*Chair: Milena Damulewicz*

**"Medical Case Reports"**
*Julia Nakoneczna, Julia Stelmach, Sandra Frycz, Julia Tubis*
*Chair: Milena Damulewicz*

**13:00-13:30** **Lunch**

**13:30-14:45** **POSTER SESSION I**

**14:45-15:00** **Gold Sponsor Presentation: I.C. Lab**

**15:00-16:00** **EXTENDED FLASH TALK SESSION: "Computational Methods in Neuroscience"**
*Chair: Michał Ślęzak, Aleksandra Domagalik-Pittner*

**16:00-17:30** **SYMPOSIA SESSION:**

**"Immunometabolic Basis of Neurodegenerative Disorders"**
*Mootaz M. Salman, Ismail Gbadamosi, Natalia Stelmach, Alonso Cerdeño-Arévalo, Violeta Belickienė*
*Chair: Ismail Gbadamosi*

**"Space-Time Trade-Offs in Human Nociceptive System"**
*Robert C. Coghill, Alexandra Mitchell, Wacław M. Adamczyk, Jakob Poehlmann, Emilia Goszczyńska*
*Chair: Wacław M. Adamczyk*

**"Interpersonal Neural Synchrony in Close Relationships: Hyperscanning Studies of Parents, Children, and Romantic Partners"**
*Antonia Hamilton, Alessandro Carollo, Weronika Bakun, Maciej Padarz, Joanna Duda-Goławska, Joanna Beck, Jan Łabędź, Grzegorz Kaliński*
*Chair: Joanna Beck*

**"Computational Methods for EEG"**
*Rosmary Blanco, Anna Grabowska, Debashis Das Chakladar, Julia Caputa*
*Chair: Mirosław Wyczesany*

**17:30-18:00** **Coffee break**

**18:00-19:00** **KEYNOTE LECTURE: "Expanding Mechanisms and Therapeutic Strategies for Neurodegenerative Disease"**
*Aaron Gitler, Department of Genetics, Stanford University, United States of America*
*Chair: Gilles van Luijtelaar*

**19:00-** **Welcome Reception**

## 25.04, SATURDAY

**8:00-9:00** **Neurofitness**
*Anna Pałasz*

**9:00-10:00** **KEYNOTE LECTURE: "Mitochondria in Brain Function and Mental Health"**
*Carmen Sandi, Laboratory of Behavioral Genetics, EPFL, Switzerland*
*Chair: Michał Ślęzak*

**10:00-11:30** **SYMPOSIA SESSION:**

**"Social and Affective Mechanisms of Psychedelics: From Acute Dynamics to Long-Term Adaptation"**
*Rebecca Böhme, Wiktoria Zaniewska, Marek Nikolič, Stanisław Adamczyk, Paweł Orłowski*
*Chair: Paweł Orłowski*

**"Microglia Meet Metabolism: Microglia as Drivers of Brain and Metabolic Health"**
*Agnes Nadjar, Patricija Čepauskytė, Weronika Tomaszewska, Jan Zakrzewski, Maria Sygidus*
*Chair: Weronika Tomaszewska*

**"Systems Neuroscience of Memory Formation"**
*Krzysztof Maćkiewicz, Joanna Yadav, Sylwia Drabik, Julia Sepielak-Świderska, Monika Puchalska, Martyna Marzec*
*Chair: Aleksandra Trenk*

**"From Seeing to Reacting: Visual Perception and Response Control"**
*Julia Papiernik-Kłodzińska, Sofiia Honcharova, Rob van der Lubbe, Paweł Basoń, Julia Klimecka*
*Chair: Renate Rutiku*

**11:30-12:00** **Coffee break**

**12:00-13:30** **SYMPOSIA SESSION:**

**"Bridging Worlds - Behavioral, Molecular and Clinical Perspectives on Psychedelics"**
*David Erritzoe, Jakub Młost, Vasiliki Skara, Wiktoria Zaniewska, Kristýna Aleksić*
*Chair: Adam Wojtas*

**"Neuroendocrine Brain"**
*Valery Grinevich, Ewelina Kałużna, Athul R Ramesh, John C. Oyem, Aleksandra Rzeszut*
*Chair: Savani Anbalagan*

**"Multimodal Brain Data Integration"**
*Sophia Ulonska, Tobias Peherstorfer, Natalia K. Freus, Łukasz Piszczek, Julian Kędys*
*Chair: Łukasz Piszczek*

**"Global Village: Electrophysiological correlates of language control"**
*Hanna Cwynar, Wiktoria Ogonowska, Kamil Wałczyk, Rafał Jończyk, András Ambrus*
*Chair: Kalinka Timmer*

**13:30-14:00** **Lunch**

**14:00-15:15** **POSTER SESSION II**

**15:15-17:00** **SYMPOSIA SESSION:**

**"Higher-Order Conditioning: From Incidental Associations to Psychotic-Like States"**
*Arnau Busquets Garcia, Melie Talaron, Unai Blanco Fundazuri, Natalia Żernicka-Glover, Irene Manzanares-Sierra*
*Chair: Arnau Busquets Garcia*

**"Novel Functional and Anatomical Imaging Approaches for the Nervous System Studies"**
*Diana Cash, Marija Petrinovic, Julia Niemczycka, Mateusz Kucharczyk, Karolina Nowalińska, Bartosz Pomierny, Kornelia Kliś, Zofia Sikorska*
*Chair: Bartosz Pomierny, Mateusz Kucharczyk*

**"Neuroscience across species"**
*Mateusz Kostecki, Svetolik Spasic, Aniqa Saiyara. Ida Ilmer, Ivaylo Iotchev*
*Chair: Mateusz Kostecki*

**"From Saccades to Saliency: The Role of Eye Movements in Sensory and Social Cognition"**
*Philippe Blondé, Katarzyna Jurewicz, Łukasz Grzeczkowski, Laureant Beaupoil*
*Chair: Marek Pędziwiatr*

**17:00-17:30** **Coffee break**

**17:30-18:30** **KEYNOTE LECTURE: "Learning Principles of Neuroscience from the 'Vulgar' Hydra"**
*Rafael Yuste, Columbia University, New York, United States of America*
*Chair: Steffen Kandler*

**19:00-** **IBRO INTEGRATION EVENT with reception, Wesoła Immersive – New Media Art Center**

## 26.04, SUNDAY

**9:30-10:30** **KEYNOTE LECTURE: "Resolving Conflict in the Brain"**
*Carolina Rezaval, School of Biosciences, Centre for Neurogenetics Research, University of Birmingham, United Kingdom*
*Chair: Ali Jawaid*

**10:30-12:00** **SYMPOSIA SESSION:**

**"Neuronal Mechanism of Social Behaviors"**
*Felix Leroy, Alicja Puścian, Paula Gómez-Sotres, Klaudia Misiołek, Netsanet Belay*
*Chair: Jan Rodriguez Parkitna, Katarzyna Starowicz*

**"Targeting Neuroinflammation and CNS Repair In MS: From Psychedelics to Oxysterols and Advanced Drug Delivery"**
*Aleksandra Rutkowska, Fatimah Zahra, Piotr Pobiarzyn, Leonardo Ricciardi, Olga Blauth*
*Chair: Piotr Pobiarzyn*

**"Neural Systems Under Strain: From Acute Functional Responses to Chronic Structural Alterations"**
*Ala Yankouskaya, Batuhan Ş. Çakır, Brianca Renfro, Mikołaj Compa, Ruzanna A. Shushanyan*
*Chair: Marianna Constantinou*

**12:00-12:30** **Coffee break**

**12:30-14:00** **SYMPOSIA SESSION:**

**"Human Cellular Approaches to Study Brain Physiology and Pathology"**
*Cristiana Cruceanu, Viorica Raluca Contu, Karolina Cierluk, Erkan Metin, Victoria Graffe*
*Chair: Viorica Raluca Contu*

**"Decoding Neuroinflammation: Immune Mechanisms, Spatial Profiling and Systemic Implications"**
*Danilo De Gregorio, Marta Kamińska, Julia Jarco, Alba Simats, Diana Piotrowska*
*Chair: Julia Jarco*

**"Sleep and Biological Rhythms: From Cognition to Clinical Practice"**
*Patrycja Ściślewska, Karolina Raczek, Anna Zofia Leśniewska, Marcin Iwański, Michał Rafał Zaręba*
*Chair: Michał Zaręba, Patrycja Ściślewska*

**14:00-14:30** **Lunch**

**14:30-15:45** **POSTER SESSION III**

**15:45-16:45** **OPEN KEYNOTE LECTURE: "Weaving Vision And Language Into Knowledge In The Human Brain"**
*Yanchao Bi, School of Psychological and Cognitive Sciences, Peking University, China*
*Chair: Ilona Kotlewska*

**16:45-** **AWARDS & CLOSING CEREMONY**
*Adrianna Zeńczak, Martyna Bernaciak (Heads of Neuronus 2026)*
*Michał Kuniecki (Neuronus Neuroscience Forum Coordinator)*

# PLENARY LECTURES

## PL.1. CONSCIOUS VISION AND ITS RESTORATION IN BLINDNESS

Pieter Roelfsema[1,2]

[1] *Netherlands Institute for Neuroscience, Amsterdam*

[2] *Institut de la Vision, Paris*

I will discuss the neural mechanisms that determine whether visual stimuli reach conscious awareness. For simple stimuli, early visual cortex mainly relays information to higher areas; once above threshold, these signals can be maintained in working memory. More complex perceptual task that require figure-ground segregation or shifts of attention depend on recurrent interactions between early visual areas and higher areas.

Understanding these mechanisms also guides strategies to restore vision when the eyes fail. A key goal is to project visual information directly onto the brain, bypassing damaged pathways. Electrical stimulation of visual cortex can evoke artificial percepts (phosphenes), even after long-term blindness. We are developing a cortical brain prosthesis with high-channel-count interfaces. By stimulating up to 1,000 electrodes, patterned activity can generate interpretable percepts, analogous to pixels forming an image.

## PL.2. EXPANDING MECHANISMS AND THERAPEUTIC STRATEGIES FOR NEURODEGENERATIVE DISEASE

Aaron Gitler

*Gitler Lab, Department of Genetics, Stanford University*

A hallmark pathological feature of the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is the depletion of RNA-binding protein TDP-43 from the nucleus of neurons in the brain and spinal cord. A major function of TDP-43 is as a repressor of cryptic exon inclusion during RNA splicing.

By re-analyzing RNA-sequencing datasets from human FTD/ALS brains, we discovered dozens of novel cryptic splicing events in important neuronal genes. Single nucleotide polymorphisms in UNC13A are among the strongest hits associated with FTD and ALS in human genome-wide association studies, but how those variants increase risk for disease is unknown.

We discovered that TDP-43 represses a cryptic exon-splicing event in UNC13A. Loss of TDP-43 from the nucleus in human brain, neuronal cell lines and motor neurons derived from induced pluripotent stem cells resulted in the inclusion of a cryptic exon in UNC13A mRNA and reduced UNC13A protein expression. The top variants associated with FTD or ALS risk in humans are located in the intron harboring the cryptic exon, and we show that they increase UNC13A cryptic exon splicing in the face of TDP-43 dysfunction.

Together, our data provide a direct functional link between one of the strongest genetic risk factors for FTD and ALS (UNC13A genetic variants), and loss of TDP-43 function. Recent analyses have revealed even further changes in TDP-43 target genes, including widespread changes in alternative polyadenylation, impacting expression of disease-relevant genes (e.g., ELP1, NEFL, and TMEM106B) and providing evidence that alternative polyadenylation is a new facet of TDP-43 pathology.

## PL.3. MITOCHONDRIA IN BRAIN FUNCTION AND MENTAL HEALTH

Carmen Sandi

*Sandi Lab – Laboratory of Behavioral Genetics, EPFL, Switzerland*

Mitochondria are emerging as important determinants of brain function and mental health. I will discuss evidence that mitochondrial properties in defined brain cell types and circuits contribute to anxiety, motivation, and stress responsiveness.

Focusing on the nucleus accumbens and related mesolimbic and prefrontal networks, I will present work showing that alterations in mitochondrial dynamics and bioenergetics in specific neuronal populations are associated with coordinated changes in transcriptional programs, neuronal structure, circuit engagement, and behavior.

Using phenotype-led approaches, cell-type-specific manipulations, single-nucleus transcriptomics, and

circuit-level analyses, I will illustrate how mitochondrial mechanisms can be identified from both naturally occurring and experimentally induced behavioral variability. I will also discuss emerging findings extending this framework beyond neurons, including evidence that astrocytic mitochondria influence blood-brain barrier properties with behavioral consequences, and that microglial mitochondrial signatures are associated with anxiety-related phenotypes.

Together, these data support the view that mitochondria participate in the regulation of core behavioral dimensions rather than representing a secondary correlate of altered brain states. I will further consider how this work informs current efforts to understand vulnerability and resilience to stress, and how it may help define biologically grounded entry points for intervention in mental health conditions, particularly those involving anxiety, motivational dysfunction, and depression.

## PL.4. LEARNING PRINCIPLES OF NEUROSCIENCE FROM THE "VULGAR" HYDRA

Rafael Yuste

*Columbia University, New York, United States of America*

The small freshwater cnidarian Hydra vulgaris has one of the simplest nervous systems, yet exhibits relatively sophisticated behaviors. Due to its transparency, its complete neural and muscle activity can be effectively imaged. Moreover, one can completely dissociate Hydra into individual cells and image how the animal reaggregates and puts itself back together.

Our goal is to take advantage of this preparation to "break the neural code" of Hydra: to understand the complete set of transformations from neural to muscle activity to behavior.

As an example of this, we have investigated somersaulting, an acrobatic locomotion, finding that it is controlled by a neuropeptide, Hym-248, secreted by a specific neuronal ensemble, using integrate to threshold algorithms in a cross-inhibition circuit motif. To further understand how Hydra's ensembles are wired, we have performed a partial ultrastructural reconstruction of the endoderm nerve net.

Neurons have several different types of vesicles, including clear and dense core ones, which could support synaptic transmission. However, most vesicles are located far from other neurons. This indicates that Hydra's endodermal nerve net operates as a non-synaptic circuit, using a neuroendocrine chemical network to implement its functional operations. To test this, we computationally reconstruct the neuropeptide-GPCRs network in Hydra and modelled its dynamics, finding that this network has a distributed and recurrent connectivity that can sustain stable functional states.

Our results suggest the existence in Hydra´s nervous systems of attractor neural networks implemented with chemical signaling, as opposed to synaptic wiring.

## PL.5. RESOLVING CONFLICT IN THE BRAIN

Carolina Rezával

*Rezaval Lab, School of Biosciences, Centre for Neurogenetics Research, University of Birmingham*

Animals constantly face situations in which competing drives demand incompatible actions. Should they pursue food or avoid danger, continue courting a mate or flee from a predator? Resolving such conflicts requires the brain to rapidly integrate sensory information with internal motivational states to select a single behavioural outcome.

Using the fruit fly Drosophila, my laboratory investigates how neural circuits prioritise behaviour when animals confront these high-stakes choices. By combining quantitative behavioural analysis with neural circuit mapping and functional imaging, we examine how social context, internal states, and prior experience reshape the neural computations that guide action selection. Our work is beginning to reveal fundamental principles by which neural circuits resolve behavioural conflict and establish priorities.

### PL.6. WEAVING VISION AND LANGUAGE INTO KNOWLEDGE IN THE HUMAN BRAIN

Yanchao Bi

*School of Psychological and Cognitive Sciences, Peking University, China*

Human brain stores tremendous amount of knowledge about this world, which is the foundation of object recognition, language, thought, and reasoning. What's the neural codes of semantic knowledge representation? Is the knowledge "roses are red" simply the memory trace of perceiving the color of roses, stored in the brain circuits within color-sensitive neural systems? What about knowledge that is not directly perceived by senses, such as "freedom" or "rationality"?

I will present a set of studies from my lab that addresses this issue, including object color (and other visual) knowledge in several populations (congenitally blind humans, color blind humans, and typically developed macaques), and semantic neural representation in individuals with early language experience deprivation. The findings point to the existence of two different types of knowledge coding in different regions of the human brain – one conservative, based on sensory experiences, and one based on language-derived machinery that support fully nonsensory information, with the latter further modulating the former.

## SYMPOSIA LECTURES

### S1.1. WHAT DOES THE BRAIN PREDICT? A CASE FOR HIGH-LEVEL PREDICTION IN THE VISUAL SYSTEM

David Richter[1,2]

[1] *Mind Brain and Behavior Research Center (CIMCYC), University of Granada, Granada, Spain*

[2] *Donders Institute for Brain Cognition and Behaviour, Radboud University Nijmegen, Nijmegen, the Netherlands*

Predictive processing accounts suggest that perception fundamentally relies on prediction and prediction error computation.

Yet it remains unclear what features, and at which level of abstraction, the brain predicts and hence computes prediction errors for.

Combining neuroimaging (EEG, fMRI) with deep neural network-based computational modelling, we show that neural responses across the visual hierarchy, including in early visual cortex, scale with high-level, but not low-level, visual surprise.

Moreover, high-level surprise influences perceptual processing rapidly, emerging around 190 ms after stimulus onset, suggesting that high-level predictions are readily integrated during perceptual inference.

These results converge with related studies in macaques and mice, supporting a feature-specific view of predictive processing in which the visual system predominantly leverages high-level predictions to guide perceptual inference rather than relying on fine-grained, low-level information. Together, these findings help constrain neurocomputational models of perceptual inference and suggest that the brain's predictive machinery is tuned to higher-order structure in sensory input.

FINANCIAL SUPPORT: Marie Skłodowska-Curie Grant (101147241) "PreVision" awarded to D.R.

### S1.2. WHEN EXPECTATIONS SILENCE THE BACKGROUND: SELECTIVE SHARPENING IN OBJECT VISION

Jakub Szewczyk

*Institute of Psychology, Jagiellonian University, Kraków, Poland*

Top-down expectations are thought to shape object representations via sharpening or dampening. Sharpening proposes that expectations boost diagnostic features of an expected object while suppressing irrelevant features, such as background objects. Dampening predicts that expected objects generate smaller prediction errors and thus reduced neural activation. Prior tests of these accounts have typically used single-object displays. Here I introduce a novel test that adjudicates between them by focusing on background objects in cluttered scenes. Participants viewed overlapping target (foreground) and non-target (background) objects while I manipulated target expectancy. Item-level encoding models quantified how strongly each background object was represented across stages of the ventral visual stream. Expectations produced sharpening-like inhibition of background features, but only when the background interfered with the task.

When the background was task-irrelevant, its features remained uninhibited across all stages. These results suggest that top-down expectations selectively sharpen object representations under competition, rather than uniformly suppressing non-target features.

FINANCIAL SUPPORT: This research is part of the project No 2022/47/P/HS6/02294 within the POLONEZ BIS programme co-funded by the National Science Centre and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 945339.

## S1.3. FILLING IN THE BLANKS: REPRESENTATION OF OCCLUDED SCENE PARTS IN EARLY VISUAL CORTEX

Mandy Viktoria Bartsch

*Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Nijmegen, the Netherlands*

Does the brain automatically fill in missing parts of a scene? Prior fMRI work shows that even when part of a scene is occluded, early visual cortex still carries information about overall scene identity.

Yet it remains unclear whether this activity reflects the specific missing content or only contextual cues from the visible parts.

To test this, we created feature-controllable scenes composed of four colored shapes. After learning these layouts behaviorally, participants performed an item-swap detection task during fMRI, with one object occluded on half of the trials.

Replicating earlier findings, we decoded scene identity from early visual cortex responses to the occluded regions alone. Critically, feature-specific models trained on color and shape also recovered information about the occluded item's features.

This shows that the brain represents missing scene elements with detailed sensory predictions, not just at the level of global scene context.

## S1.4. SEMANTIC REPRESENTATIONS IN THE VISUAL CORTEX OF BLIND AND SIGHTED HUMANS

Łukasz Bola[1,2]

[1] *Institute of Psychology, Jagiellonian University, Kraków, Poland*

[2] *Center for Brain Research, Jagiellonian University, Kraków, Poland*

What is the function of the visual cortex when deprived of visual input? Research on this question shows that in blind individuals the "visual" areas respond to linguistic stimuli, such as words and sentences. This may indicate that, in the absence of vision, the visual cortex becomes recruited for high-level computations that are atypical for this region. Alternatively, the lack of visual input may uncover typical (i.e., present also in sighted individuals) representations of linguistic stimuli in this area.

In this talk, I will describe the results of three studies aimed at disentangling these competing hypotheses.

Sighted and blind participants were presented with concrete, abstract, and pseudo words while undergoing fMRI. We used multi-voxel pattern analysis to probe representations elicited in the visual areas in both groups during word processing.

The results suggest that, during word processing, the blind visual cortex represents a specific semantic dimension: the knowledge about physical properties of word referents. In line with the "uncovering hypothesis", we found representations of the same dimension in the sighted visual cortex. Notably, however, in sighted individuals these representations seemed more salient in high-level visual areas, whereas in blind individuals they were robust in both low-level and high-level regions.

These findings suggest that at least some of the responses to linguistic stimuli in the blind visual cortex can be driven by mechanisms that are also present in the sighted adult brain. In sighted individuals, the physical properties of word referents might be back-projected to the visual system to predict incoming visual information, initiate visual imagery, and support visuospatial thinking. In blind individuals, this mechanism might be preserved and, combined with increased sensitivity of the visual cortex in this population, drive language-related responses in this region.

FINANCIAL SUPPORT: A National Science Center Poland grant (2020/37/B/HS6/01269), a Polish National Center for Academic Exchange fellowship (BPN/SEL/2021/1/00004), and an ERC Starting Grant (BLINDBRAIN, 101164042).

## S2.1. THE ARCHITECTURE OF PAIN: MOLECULAR AND ANATOMICAL INSIGHTS INTO THE ANTEROLATERAL SYSTEM

Andrew Bell[1,2]

[1] *School of Psychology and Neuroscience, University of Glasgow, Glasgow, United Kingdom*
[2] *School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, United Kingdom*

The anterolateral system (ALS) is a major ascending pathway from the spinal cord that projects to multiple brain areas. Activity in the projection neurons of the ALS underlies the perception of pain, itch, and skin temperature, and this tract therefore represents an attractive target for novel analgesics. Despite its importance, our understanding of this system has been hampered by the considerable functional and molecular diversity of its constituent cells. In particular, we have a poor understanding of how different classes of neuron within this heterogeneous pathway transmit diverse sensory modalities and drive the dimensionality of pain. Dr Andrew Bell will present recent work using single nucleus RNA sequencing to reveal the molecular architecture of the ALS in mice. The session will explore how to target specific neuronal subsets of these cells in transgenic mice and how this can permit investigation of their distinct projectome and function.

## S2.2. GATING THE PERIPHERY: DESCENDING CONTROL OF SENSORY INPUT IN PAIN AND CANCER

Mateusz Kucharczyk[1,2,3]

[1] *Cancer Neurophysiology Research Group, Łukasiewicz Research Network–PORT Polish Centre for Technology Development, Wrocław, Poland*
[2] *Biophotonics and Electrophysiology Laboratory, Center for the Development of Therapies for Civilization and Age-Related Diseases, Jagiellonian University Medical College, Kraków, Poland*
[3] *Wolfson Sensory, Pain, and Regeneration Centre, King's College London, London, United Kingdom*

Descending neuronal pathways shape somatosensation by regulating spinal circuits and primary sensory neuron activity, enabling the brain to modulate afferent signal flow and influence peripheral tissues through neurogenic mechanisms. Our laboratory integrates somatosensory neuroscience with cancer biology to investigate how these pathways contribute to tumorigenesis and cancer-associated pain. Using in vivo electrophysiology and calcium imaging combined with selective opto- and chemogenetic modulation of genetically and anatomically defined circuits, we record spinal and peripheral neuronal activity and relate it to behaviour through machine-learning–assisted analysis. This systems-level approach aims to link network-wide dynamics with top-down control of nociception and tumour progression.Dr. Kucharczyk will illustrate how descending pathways regulate spinal projection neurons and presynaptic terminals of primary afferents, including peptidergic and silent nociceptors in cancer-induced bone pain. He will also discuss neurogenic inflammation and introduce fiber-type–specific functional imaging strategies that reveal new opportunities to modulate nociceptive transmission through defined spinal and supraspinal pathways.

## S2.3. HIERARCHICAL ORGANIZATION OF MECHANO-NOCICEPTIVE PATHWAYS REVEALED BY ACTIVITY LABELING

Gabriela Basile Carballo, Felipe Meira de Faria, Melisa Maidana Capitan, Ilona Szczot, Marek Brodzki, Christoffer Karlson, Leandro Flores do Nascimento, Håkan Olausson, Max Larsson, Marcin Szczot

*BKV/CSAN, Linköpings Universitetet, Linköping, Sweden*

Nociception safeguards organisms from external injury and internal tissue damage by detecting and distinguishing harmful stimuli, and encoding their intensity and location, thereby driving adaptive behavioral and homeostatic responses.However, the relationship between specific function and molecular identity across nociceptors remains poorly understood.

Here,we mapped the peripheral architecture of nociceptive signaling by combining in vivo activity labeling of pelvic nerve afferents with single-cell RNA sequencing to understand how different classes of nociceptors encode interoceptive signals.

To create a reference atlas,freshly dissociated cells in L5-S1 DRGs from adult C57BL/6Jrj mice were manually collected and sequenced using the Smart-Seq3 method.To label noxious stimuli-activated cells,we used animals that expressed the photoconvertible calcium reporter CaMPARI2A series of nociceptive stimuli was delivered,while DRGs were exposed to conversion-inducing UV-light,fluorescently labeling activated neu-

rons.Labelled cells were sequenced and embedded in the control cell atlas.

We developed a cell atlas with 1037 manually sorted neurons and detected 10,000 genes per cell.Sequencing of 2.997 labelled cells revealed that while nociceptive stimuli activated multiple neuronal classes,some were specific to visceral nociceptive signals.For C- nociceptors, Adra2a cells were abundant among colon-innervating neurons but nearly absent among skin-innervating populations.A-nociceptors showed clear target-specific specialization with Adm cells preferentially responding to colorectal stroke and anorectal distension. Moreover,we identified a subpopulation of Adm neurons, marked by the Uts2b+ gene, that relates to physiological bladder stimulation specifically.

These findings reveal a hierarchical organization of peripheral mechanical pain encoding, in which increasingly specialized mechano-nociceptor populations are differentially engaged according to tissue domain and organ context.

## S2.4. BRIDGING IDENTITY AND FUNCTION OF VAGINAL AFFERENTS UNCOVERS C-LTMR CONTROL OF FEMALE SEXUAL BEHAVIOR

Marek Brodzki[1], Leandro Flores do Nascimento[1], Felipe Meira de Faria[1], Gabriela Basile Carballo[1], Melisa Beatriz Maidana Capitán[1], Ilona Szczot[1], Vasiliki Lida Lerou[1], Yaren Sert[1], Max Larsson[2], Marcin Szczot[1]

*[1] Center for Social and Affective Neuroscience, Department of Clinical and Biomedical Sciences, Linköping University, Linköping, Sweden*

*[2] Division of Cell and Neurobiology, Department of Clinical and Biomedical Sciences, Linköping University, Linköping, Sweden*

Sensation of touch has a complex role in sexual behavior, as it carries both tactile and emotional information, which is underlined by extremely high innervation density of genital organs by touch neurons. While previous reports indicate particular neuron types to be important for sexual behavior, the general organization of how the rich sexual experience is encoded across molecularly defined neuronal classes is unknown, especially in females.

This study aims to elucidate the functional landscape of cells that innervate female genitalia and to pinpoint the molecularly defined neuronal types that sense and control behavioral responses that are vital for reproduction.

We use anatomical assessment of nerve endings of perineal skin and genitals, in vivo calcium imaging in transgenic mice, functional labelling of neurons activated by naturalistic vaginal stimulation combined with single-cell RNA sequencing, and sexual behavior assays in animals with conditional neurotransmission block.

We find that the murine female genital organs are innervated by both myelinated and unmyelinated fibers, but it is the C-low threshold mechanoreceptors (C-LTMRs) that are most robustly responding to the stimulation of the perineum and the inside of the vagina. Moreover, we show that these neurons directly regulate behavior of female mice during coitus, by regulating their sexual receptivity.

Together, our findings identify C-LTMRs as key mediators of vaginosensation that regulates sexual behavior. These results provide a comprehensive survey of vaginal innervation and help understand the neural basis of touch that underlies mammalian reproduction.

FINANCIAL SUPPORT: Swedish Research Council (Vetenskapsrådet 2024-362 02781), Brain Foundation (Hjarnfonden 2025-00169).

## S2.5. WHEN BODY-BRAIN COMMUNICATION GOES AWRY: EMBODIMENT OF EMOTIONS AND STATES IN NOCIPLASTIC PAIN

Aleksandra Herman

*Laboratory of Brain Imaging, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland*

Pain is a complex, multisensory phenomenon with sensory, emotional, and motivational components. Nociplastic pain, a type of chronic pain, arises from altered nociception without clear tissue damage or nervous system lesions. In these conditions, the brain may misinterpret body signals, making pain maladaptive. Little is known, however, about whether altered body-brain communication extends to misattributing emotions as pain.

Using a cross-sectional design, we investigated how individuals with nociplastic pain perceive, identify, and interpret emotions and bodily sensations compared to pain-free controls.

Across two studies, individuals with nociplastic pain and matched pain-free controls completed the emBODY task, mapping bodily sensations for emotional, neutral, pain-, and fatigue-related states. Participants also com-

pleted measures of alexithymia, bodily sensation interpretation, and interoception.

Linear discriminant analysis showed that classification accuracy of body sensation maps was lower in the nociplastic pain group. Compared with controls, they had higher alexithymia, greater awareness of bodily signals, more negative interpretations of ambiguous sensations, and self-reported interoceptive difficulties.

Findings indicate amplified perception of bodily signals, reduced differentiation of emotions and non-emotional states, and interoceptive difficulties in nociplastic pain. Interventions targeting perception, differentiation, and interpretation of bodily sensations may improve pain symptoms and daily functioning.

FINANCIAL SUPPORT: European Commission (EC), Horizon Europe Framework Programme (MSCA) no 101059716; NCN Sonata 2023/51/D/HS6/00364.

## S3.1. WHAT CAN WE LEARN ABOUT PARKINSON'S DISEASE FROM FRUIT FLIES?

Alex Whitworth

*MRC Mitochondrial Biology Unit, University of Cambridge, UK*

Parkinson's disease (PD) is a common neurodegenerative movement disorder that is typically caused by a combination of genetic and environmental factors. Relatively rare inherited forms of PD have delivered incredible insights into the pathogenic mechanism and have consistently highlighted defects in proteostasis and mitochondrial dysfunction. Two genes linked to inherited PD, PINK1 and PRKN, provide the strongest links to mitochondrial dysfunction as their encoded proteins (the kinase PINK1 and ubiquitin ligase Parkin) function to signal the selective degradation of mitochondria – a process termed mitophagy. Many aspects of PINK1/Parkin function are deeply conserved through evolution. Since their development, the Drosophila models of the orthologous genes, Pink1 and parkin, have delivered fundamental insights into their physiological function. The strength of the model is due largely to the robust phenotypes of the genetic knockouts which confer mitochondrial disruption, locomotor deficits and neurodegeneration under basal conditions. Applying the powerful genetic tools available in Drosophila has uncovered important molecular and cellular aspects of Pink1/parkin disruption to tissue and organismal health. A key advantage of analysing Pink1/parkin function in an animal model is the ability to interrogate inter-organ, such as the gut-brain axis, as well as systemic impacts on neurodegeneration and healthspan. Here I will present our latest advances on understanding the physiological roles of Pink1/parkin as well as the consequences of their dysfunction and therapeutic opportunities.

## S3.2. THE LINK BETWEEN CIRCADIAN CLOCK AND PARKINSON'S DISEASE DEVELOPMENT – RESEARCH ON DROSOPHILA MELANOGASTER MODEL

Justyna Kadłuczka, Milena Damulewicz

*Institute of Zoology and Biomedical Research, Department of Biology and Cell Imaging, Kraków, Poland*

Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons within the substantia nigra pars compacta. Although PD is primarily described as a motor disorder, prodromal symptoms such as circadian clock desynchronization, sleep disturbances, and blurred vision may occur years before diagnosis. It has been implicated that non-motor symptoms, especially circadian clock disruption, can exacerbate the progression of PD.

We investigated how mitophagy disruption or the expression of human α-synuclein exclusively in photoreceptors affected retinal function and whether the observed changes might contribute to the development and progression of PD.

In our study, we used a genetic model of PD in Drosophila melanogaster either by silencing park gene or expressing human α-synuclein in the visual system. We performed behavioral analysis: climbing assay, sleep and locomotor activity. We analyzed the external structure of the Drosophila ommatidia using scanning electron microscopy and examined the morphology of the photoreceptors with histological staining. Finally, to determine the number of dopaminergic neurons we immunostained for tyrosine hydroxylase.

We observed that mitophagy disruption reduced the flies' overall fitness and affected both daytime and nighttime sleep. Synucleinopathy not only altered sleep pattern but also severely disrupted photoreceptor morphology and reduced the number of dopaminergic neurons.

Our data revealed that inducing PD in the visual system affected processes that, in Drosophila, are regulated by distinct central brain structures.

FINANCIAL SUPPORT: Supported by the National Science Centre grant SONATA BIS-12 K/NCN/000167

## S3.3. GBA2 INHIBITORS AS A NEW APPROACH TO TREATING NEURODEGENERATIVE DISEASES

Zuzanna Kula[1], Danielle Te-Vruchte[2], Frances Platt[2], Carlo Breda[1], Daniel Sillence[1]

[1] *De Montfort University, School of Pharmacy, The Gateway, Leicester, United Kingdom*

[2] *University of Oxford, Department of Pharmacology, Mansfield Road, Oxford, United Kingdom*

Mutations in GBA1, encoding the lysosomal hydrolase, represent the most common genetic risk factor for Parkinson's disease (PD). The prevailing model proposes that mutant GBA1 is inactive, leading to glucosylceramide (GlcCer) accumulation. However, clinical worsening following GlcCer synthesis inhibition suggests that GlcCer is required for cellular function. We hypothesise that misfolded mutant GBA1 is retained in the ER, where it acquires GBA2-like activity and depletes cytoplasmic GlcCer pools. As these pools regulate v-ATPase activity and lysosomal acidification, their loss may impair lysosomal and synaptic vesicle function, disrupting dopamine handling. Given shared lysosomal mechanisms, this pathway may extend to Alzheimer's disease (AD).

To determine whether modulation of cytoplasmic GlcCer pools restores lysosomal function in GBA1 mutant cells and improves neurodegenerative phenotypes in PD and AD models.

Human N370S and L444P fibroblasts were treated with adamantyl GlcCer, and lysosomal pH and volume were quantified. In vivo, Drosophila melanogaster expressing human GBA1 N370S or L444P, or the APP C-terminal fragment (AD model), were treated with a GBA2 inhibitor (1 mM) throughout adulthood. Lifespan and motor function (negative geotaxis) were assessed.

Adamantyl GlcCer significantly rescued lysosomal pH and reduced lysosomal enlargement in N370S fibroblasts, supporting a functional role for ER/cytoplasmic GlcCer pools. In Drosophila, GBA2 inhibition significantly prolonged lifespan and improved climbing ability in GBA1 mutant flies. Similarly, AD model flies showed restoration of motor performance to near wild-type levels following treatment.

These findings support a revised model in which cytoplasmic GlcCer depletion, rather than storage, contributes to neurodegeneration. Restoring GlcCer homeostasis via GBA2 inhibition improves lysosomal and neuronal function across PD and AD models, highlighting GlcCer regulation as a promising therapeutic target.

FINANCIAL SUPPORT: De Montfort University Bursary.

## S3.4. ACCELERATED DOPAMINERGIC DYSFUNCTION AND PARKINSONIAN BEHAVIOUR IN ADULT ZEBRAFISH FOLLOWING OPTIMIZED ROTENONE EXPOSURE

Chin Long Poo[1,2], Nur Liana Md Nasir[1], Nik Aina Syazana Nik Zainuddin[1], Mohd Rahimi Ashraf Abd Rahman[1], Siti Soleha Binti Ab Dullah[1], Mei Siu Lau[1], Siti Khadijah Mustapha Kamal[1], Elda Nurafnie Binti Ibnu Rasid[1], Muhammad Lokman Md Isa[2], Hussin Muhammad[1]

[1] *Toxicology & Pharmacology Unit, Herbal Medicine Research Centre, Institute for Medical Research, National Institutes of Health, Selangor, Malaysia*

[2] *Institute of Planetary Survival for Sustainable Well-being, International Islamic University Malaysia, Pahang, Malaysia*

Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by the progressive loss of dopaminergic neurons in the substantia nigra and motor impairment. PD can be induced in vivo using specific neurotoxic chemicals. While rotenone is widely used to induce PD-like phenotypes in adult zebrafish, commonly reported protocols employ lower concentrations over prolonged exposure periods, resulting in variability in disease onset and experimental timelines.

This study aims to establish a faster and more consistent rotenone-induced PD model in adult zebrafish using an optimized exposure concentration.

Adult zebrafish were exposed to rotenone at 5 µg/L and 7.5 µg/L for a total of 4 weeks. Locomotor and anxiety-related behaviours were assessed longitudinally from Day 3 to Day 28. Dopamine levels were measured on the last day of assessment.

Zebrafish exposed to 5 µg/L rotenone exhibited Parkinsonian-like motor deficits by Day 19, whereas exposure to 7.5 µg/L induced significant and consistent reductions in total distance travelled and mean swimming speed as early as Day 12. In addition, increased bottom-dwelling behaviour and freezing duration were observed earlier and more robustly in the 7.5 µg/L group, indicating accelerated development of PD-like

behavioural phenotypes. Dopamine analysis performed at the late stage of exposure further confirmed dopaminergic impairment in rotenone-treated fish, supporting the observed behavioural deficits. The 7.5 µg/L exposure paradigm produced a stable and reproducible PD-like phenotype within a shorter timeframe compared with the widely established 5 µg/L long-term exposure model.

In conclusion, chronic exposure to rotenone at 7.5 µg/L represents a faster and reliable method for inducing Parkinson's disease-like phenotypes in adult zebrafish, offering a practical and efficient platform for mechanistic studies and neuroprotective drug screening.

FINANCIAL SUPPORT: This research was funded by National Institutes of Health, Ministry of Health, Malaysia.

## S3.5. ASTROCYTE-SECRETED FACTORS AS KEY PLAYERS IN THE POST-ISCHEMIC BRAIN

Aleksandra Klachacz[1], Aleksandra Tempes[1], Magda Bakun[2], Leif T. Koschützke[3], Amido Daugardt[3], Christoph Harms[3], Anna R. Malik[1]

*[1] Cellular Neurobiology Research Group, Institute of Developmental Biology and Biomedical Sciences, Faculty of Biology, University of Warsaw, Poland*
*[2] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland*
*[3] Department of Experimental Neurology, Charité-Universitätsmedizin Berlin, Germany*

Ischemic stroke is a major cause of death and long-term disability, affecting millions worldwide, with a significant proportion of people experiencing persistent motor deficits. Astrocytes are key regulators of the brain environment and become activated in response to ischemic injury. Upon activation, they release a wide spectrum of factors responsible for modulating inflammation, extracellular matrix (ECM) remodeling, and tissue repair.

The study aimed to characterize the astrocytic secretome under post-ischemic conditions and to identify mechanisms regulating the expression and secretion of ECM-related proteins and, most importantly – CHI3L1.

To investigate the astrocytic secretome after stroke, we combined an in vitro model mimicking post-stroke astrocyte activation with mass-spectrometry analyses. Key findings were validated using an in vivo stroke model in mice as well as various in vitro strategies for astrocyte activation.

Proteomic profiling revealed a strong enrichment in ECM-related proteins and elevated levels of CHI3L1 in the secretome of activated astrocytes. Subsequent analyses demonstrated that TGFβ and proinflammatory factors such as IL1β and TNFα upregulate CHI3L1 expression and secretion in astrocytes.

Our ultimate goal is to define how factors secreted by astrocytes not only shape the brain microenvironment, but also influence regeneration and recovery after stroke. The results obtained so far indicate that CHI3L1 is a dynamically regulated component of the reactive astrocytes secretome with its expression and secretion upregulated by TGFβ and pro-inflammatory cytokines.

FINANCIAL SUPPORT: National Science Centre, SONATA BIS 14 2024/54/E/NZ4/00134 (Project leader: Anna Malik, PhD DSc, associate professor).

## S4.1. SUBARACHNOID HEMORRHAGE: IS IT THE ONLY CAUSE?

Julia Nakoneczna[1], Michał Krakowiak[2]

*[1] Medical University of Gdańsk, Studenckie Koło Naukowe Neurochirurgii, Gdańsk, Poland*
*[2] Medical University of Gdańsk, Department of Neurosurgery, Gdańsk, Poland*

Posterior fossa arteriovenous malformations are rare, representing only 5–15% of all AVMs, with an incidence in the population of 2 per 10,000. Because of the presence of vital structures in this confined area, AVMs of posterior fossa are life-threatening. We present the case of a 31-year-old female patient, admitted for a subarachnoid haemorrhage with atypical presentation in whom angiography showed a Spetzler–Martin grade 3 AVM associated with AICA treated with embolization.

Our aim is to highlight the surgical and diagnostic difficulties, review diagnostic possibilities and summarize the management options.

The study is a retrospective case report. Clinical, laboratory, and imaging data were obtained from the patient's medical records. Diagnostic procedures were performed according to standard clinical practice.

The case provides an excellent illustration of the importance of thorough analysis. Although the reported headache does not in any way suggest or evoke the possibility of subarachnoid hemorrhage, it turned out

that the patient was admitted to the department with precisely this clinical presentation. On the other hand, there are two peaks in age at presentation of AVM, one in childhood and another at age 30 to 50, into which our patient fits perfectly, as her SAH originated from an AVM. This highlights the importance of a multidimensional perspective when evaluating such cases. A second highly significant aspect emphasized by this case is the crucial role of grading scales in determining the optimal treatment strategy.

The case underlines several key points. Firstly, the need for accurate diagnostic imaging, preferably including thin-section MRI, to improve the detection of small vessel abnormalities and guide therapeutic planning. Secondly, the central role of angioarchitecture and Spetzler–Martin grading in treatment selection, and thirdly, the fact that not all lesions require immediate intervention, as some may undergo spontaneous regression.

FINANCIAL SUPPORT: There was no financial support provided.

## S4.2. DURAL ARTERIOVENOUS FISTULA MIMICKING CAROTID CAVERNOUS FISTULA: AN ATYPICAL CAUSE OF OCULAR SYMPTOMS – A CASE REPORT

Julia Stelmach[1], Patrycja Jaworska[1], Jakub Rychlik[1], Michał Krakowiak[2]

[1] *Medical University of Gdańsk, Student's Scientific Circle of Neurosurgery, Gdańsk, Poland*

[2] *Medical University of Gdańsk, Department of Neurosurgery, Gdańsk, Poland*

Cranial dural arteriovenous fistulas (dAVFs) are rare vascular anomalies characterized by abnormal connections between dural arteries and venous sinuses or cortical veins. Their clinical manifestations depend primarily on venous drainage patterns. Although carotid cavernous fistulas (CCFs) are classically associated with ocular symptoms such as chemosis, proptosis, and elevated intraocular pressure, similar presentations caused by dAVFs are uncommon and may lead to misdiagnosis.

To present a case of a cranial dAVF mimicking a carotid cavernous fistula and to highlight the importance of accurate differential diagnosis in patients presenting with ocular symptoms suggestive of CCF.

We report the case of an 83-year-old male with a 4-month history of progressive left-sided exophthalmos, lacrimation, and conjunctival redness. Initial imaging suggested a carotid cavernous fistula. However, detailed digital subtraction angiography (DSA) was performed to further evaluate the vascular lesion and determine the exact angioarchitecture and venous drainage pattern.

Angiographic studies revealed multiple dural arteriovenous fistulas draining into the left sigmoid sinus with retrograde cortical venous reflux, classified as Cognard IIb and Borden II. The patient underwent endovascular embolization using Onyx 18, which resulted in complete occlusion of the fistulas. The postoperative course was uneventful, and the patient was discharged in good clinical condition without neurological deficits.

This case underscores the necessity of including dAVFs in the differential diagnosis of patients presenting with ocular symptoms typically attributed to CCFs. Failure to recognize a dAVF with retrograde cortical venous drainage may delay treatment and increase the risk of hemorrhage or neurological deterioration. Comprehensive angiographic evaluation and early endovascular intervention are crucial for achieving favorable clinical outcomes.

## S4.3. EEG SIGNATURES OF LATE-STAGE AMYOTROPHIC LATERAL SCLEROSIS RESEMBLE PATTERNS CHARACTERISTIC FOR UNRESPONSIVE WAKEFULNESS SYNDROME

Sandra Frycz[1,2], Wiktor Więcławski[1,2], Marcin Skotniczny[1,2], Marek Binder[1]

[1] *Institute of Psychology, Jagiellonian University, Kraków*

[2] *Doctoral School of Social Sciences, Jagiellonian University, Kraków*

Amyotrophic lateral sclerosis (ALS) is a progressive, incurable neurodegenerative disease characterized by loss of motor neurons, leading to muscle weakness, respiratory failure, and death. Although the cerebral cortex has traditionally been considered relatively preserved, accumulating evidence indicates that cognitive impairment may occur in a substantial proportion of patients. This suggests neurodegeneration may extend beyond the motor system and involve cortical networks, potentially contributing to altered consciousness and EEG patterns resembling unresponsive wakefulness syndrome (UWS) – a syndrome defined by wakefulness without awareness.

This case study evaluated auditory system functioning and covert consciousness using electrophysiological measures in a 45-year-old woman diagnosed with ALS six years prior to assessment.

The protocol included resting-state (RS) EEG, 40-Hz auditory steady-state responses (ASSR), and a passive auditory oddball paradigm. Before EEG recording, auditory screening was performed, including otoacoustic emissions and auditory brainstem responses.

RS EEG was dominated by 1–3 Hz delta oscillations with an undifferentiated 1/f spectrum, predominantly over prefrontal regions, resembling frontal intermittent rhythmic delta activity. No event-related responses were detected in the oddball paradigm, and ASSR responses were absent. Auditory testing revealed no measurable otoacoustic or brainstem responses.

Findings indicate severe cortical dysfunction and provide no electrophysiological evidence of covert consciousness. The neurophysiological profile is consistent with a clinical presentation resembling UWS in end-stage ALS.

FINANCIAL SUPPORT: The research was supported by National Science Centre (2024/53/B/HS6/04255).

## S4.4. SEVERE SERONEGATIVE MILLER FISHER SYNDROME COMPLICATED BY ACUTE RESPIRATORY FAILURE: A CASE REPORT

Julia Tubis, Agnieszka Stankowska, Olga Kurzych, Jacek Gozdalski
*University Clinical Centre, Department of Adult Neurology, Gdańsk, Poland*

Miller Fisher syndrome (MFS) is a rare variant of Guillain-Barre syndrome characterized by ophthalmoplegia, ataxia, and areflexia. Although MFS typically has a mild clinical course , severe life-threatening presentations can occur.

The aim is to present an unusual, life-threatening case of seronegative MFS complicated by rapid respiratory failure, highlighting the crucial role of clinical evaluation when laboratory confirmation is absent.

A 48-year-old male presented with dysarthria, gait ataxia, and diplopia , preceded by fever and cough. Examination revealed bilateral ophthalmoplegia, four-limb ataxia, and areflexia. Initial cerebrospinal fluid analysis and head magnetic resonance imaging were unremarkable. Electroneurography showed axonal-demyelinating damage with conduction blocks. Ganglioside immunoblot antibodies were negative.

MFS diagnosis was established based on the clinical presentation. The patient developed acute respiratory failure requiring endotracheal intubation , mechanical ventilation , and a subsequent tracheostomy. Treatment included intravenous immunoglobulins and antibiotics for concurrent pneumonia. Over the following weeks, significant clinical improvement was observed. The patient was successfully decannulated and regained independent ambulation.

While MFS is generally considered a benign condition, clinicians must remain vigilant for atypical courses involving rapid respiratory decline. Furthermore, a severe clinical manifestation can occur in seronegative patients, where diagnosis must rely strictly on the clinical evaluation. Prompt intensive care and timely immunotherapy are crucial.

FINANCIAL SUPPORT: None.

## S5.1. TARGETING THE BLOOD-BRAIN BARRIER: NEW FRONTIERS IN NEURODEGENERATION AND BRAIN HEALTH

Mootaz M. Salman
*Univeristy of Oxford, United Kingdom*

Neurodegenerative diseases are multifactorial and heterogeneous conditions and leading cause of morbidity and mortality. Our work aims to answer the question: how does inflammation-mediated blood-brain barrier (BBB) dysfunction lead to the development of neurodegeneration and whether we can stop it? Increasing evidence supports the involvement of BBB dysfunction in neurodegenerative disorders including Parkinson's, Alzheimer's and small vessel dementia; it is evident that this dysfunction happens even before the onset of dementia. In-depth understanding of the cell-cell interactions and signalling pathways between the core elements of the BBB will help in defining and validating new therapeutic targets for the prevention of dementia. In our work, we developed advanced microfluidic 3D BBB-on-a-chip models using patient-derived iPSCs and human primary cells and identified a number of molecular targets that contribute to barrier integrity and function in astrocytes and pericytes. Our work provides new tools to understand lifelong brain health, describe the basis of BBB dysfunction in the occurrence and development of neurodegeneration, and provides a platform to develop new treatments for dementia and related CNS pathologies.

## S5.2. TDP-43-METABOLISM INTERPLAY IN MOTOR NEURONS: IMPLICATIONS FOR ALS PATHOPHYSIOLOGY

Ismail Gbadamosi[1], Jan Zakrzewski[1], Ilke Guntan[1], Dorota Dymkowska[2], Ali Jawaid[1]

[1] *Translational Neuropsychiatry Research Group, Life Sciences and Biotechnology Center, Lukasiewicz Research Network–PORT Polish Center for Technology Development, Warsaw, Poland*

[2] *Laboratory of Cellular Metabolism, Nencki Institute of Experimental Biology, Warsaw, Poland*

TDP-43 nuclear depletion and cytoplasmic mislocalization are defining molecular features of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Epidemiological evidence points to critical roles for metabolic cascades in ALS and PTLD pathogenesis, yet the role of TDP-43 in regulating brain energy metabolism remains unclear.

We aimed to examine the effects of TDP-43 dysfunction on motor neuronal and microglial metabolism and its functional consequences for neuronal health.

TDP-43 was depleted in mouse motor neuron- and microglia-like cells using RNA interference, followed by assessment of glycolytic flux, mitochondrial oxidative phosphorylation (OXPHOS), and cellular energy sensing.

TDP-43 loss induced divergent metabolic remodeling in a cell-type-specific manner. In motor neurons, TDP-43 depletion triggered a hypermetabolic state characterized by coordinated upregulation of glycolysis and mitochondrial OXPHOS, accompanied by impaired AMPK signalling, leading to sustained energetic demand. In contrast, microglia underwent a pronounced shift toward glycolysis without a corresponding increase in mitochondrial OXPHOS. This glycolytic reprogramming was functionally consequential, promoting exaggerated engulfment of healthy synaptoneurosomes. Importantly, bypassing glycolysis rescued the enhanced engulfment phenotype, supporting the notion that altered energy metabolism is a causal driver of pathological microglial behavior.

Collectively, these findings identify TDP-43 dysfunction as a driver of cell-type-specific metabolic rewiring, coupling neuronal hypermetabolism and impaired energy sensing with microglial glycolytic activation and maladaptive synaptic pruning. This neuron–microglia metabolic mismatch provides a mechanistic pathway by which TDP-43 pathology can cause both motor neuron stress and non-cell-autonomous synaptic injury, highlighting energy metabolism as a tractable therapeutic axis in ALS and FTD.

FINANCIAL SUPPORT: This study is supported by the Polish Academy of Science.

## S5.3. RATIONAL DESIGN OF SUBUNIT-SELECTIVE IMMUNOPROTEASOME INHIBITORS RELEVANT TO NEUROINFLAMMATION

Natalia Stelmach[1], Paulina Hładki[2], Wioletta Rut-Schedlok[1], Katarzyna Popiołek-Barczyk[3], Natalia Małek[1]

[1] *Wrocław University of Science and Technology, Department of Chemical Biology and Bioimaging, Poland*

[2] *Poznań University of Technology, Institute of Computing Science, Poznań, Poland*

[3] *Polish Academy of Science, Maj Institute of Pharmacology, Kraków, Poland*

Dysfunction of the proteasome and immunoproteasome (i20S) contributes to proteotoxic stress, chronic microglial activation and neuroinflammation in neurodegenerative disorders and chronic pain conditions. In previous work, we demonstrated that LPS-stimulated microglia and distinct brain regions of animals in the chronic constriction injury (CCI) model undergo strong i20S remodeling with upregulation of β1i, β2i, and β5i and changes in catalytic activity and gene expression. These findings underscore the need for subunit-selective chemical tools to dissect the immunoproteasome's role in CNS pathology.

Here, we combined this biological framework with computational approach to design selective peptide-based inhibitors.

Using large libraries of natural and non-natural amino acids, we mapped positional preferences (P2–P6) across six catalytic β subunits (β1/β1i, β2/β2i, β5/β5i) and identified optimal residues that define subunit-specific recognition motifs. This enabled the rational construction of ideal peptides predicted to exhibit maximal selectivity.

Docking analyses using AutoDock Vina revealed distinct pocket geometries and interaction networks that explain subunit-selective binding and provide a structural basis for targeted inhibitor design.

Together, this framework links immunoproteasome biology with structure-guided design and offers new avenues for developing selective proteasome modulators relevant to neurodegeneration.

FINANCIAL SUPPORT: The study is supported by National Science Center, Poland, grant OPUS 2023/49/B/NZ7/02172.

## S5.4. COMPARATIVE PROTEOMICS OF THE OCULOMOTOR REGION AND THE VENTRAL SPINAL CORD: IDENTIFYING MOLECULAR SIGNATURES UNDERLYING NEURONAL RESILIENCE

Alonso Cerdeño-Arévalo[1], Elena Anaya-Cubero[2], Enrique Santamaría[2], Rebeca Mejías[1,3], Ángel Manuel Pastor[1]

[1] *Departamento de Fisiología, Facultad de Biología, Universidad de Sevilla, Spain*

[2] *Clinical Neuroproteomics Laboratory, Navarrabiomed, HUN-UPNA-IDISNA, Pamplona, Spain*

[3] *Instituto de Biomedicina de Sevilla (IBIS), CSIC-Universidad de Sevilla, Spain*

Amyotrophic Lateral Sclerosis (ALS) and Muscular Spinal Atrophy (MSA) lead to the degeneration of motor neurons. Despite their distinct aetiologies and clinical progression, neurons in the oculomotor (OCM) region display remarkable resilience compared to other motor neuron populations. Previous transcriptional studies suggest that differential motor neuron vulnerability may arise from celltypespecific gene regulation and inherent molecular differences that modulate cellular responses to disease.

In this work, we investigate these differences at the protein level by comparing the "resilient" oculomotor (OCM) region with the "vulnerable" spinal cord (SC), aiming to identify potential neuroprotective molecular signatures.

Fresh frozen tissue from p63 ChAT-IRES-Cre::Ai9 (RCL-tdT) mice (n=12, 6 males and 6 females) was processed for LC-MS/MS analysis. Raw LFQ data were annotated using MaxQuant, and downstream statistical analyses were performed using Perseus v1.6.15. Enrichment analyses were performed using both publicly available and licensed software tools, including Metascape, Ingenuity Pathway Analysis (IPA), and curated pipelines for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations using R.

Comparative proteomics analysis of the OCM and the SC revealed significant enrichment of pathways related to synapse organization, regulation, and signaling. In contrast, metabolic pathways associated with mitochondrial function and lipid metabolism were markedly downregulated in the OCM. Notably, molecules linked to motor neuron degeneration, such as SOD1 and SOD2, were upregulated in the OCM region.

The OCM region displays a unique protein signature linked to lipid and mitochondrial metabolism, and elevated antioxidant expression relative to the SC. These pathways may contribute to the intrinsic resilience of OCM neurons, in contrast to the vulnerability observed in spinal motor neuron populations affected in ALS and SMA.

FINANCIAL SUPPORT: Supported by: I+D+i project PID2021-124300NB-I00 and PID2024-155943NB-I00 of MICIU/AEI/10.13039/501100011033/ FEDER, UE; and by P20_00529 by Junta de Andalucía-FEDER, PREDOC PAID2020.

## S5.5. CIRCULATING YRNAS AS NOVEL BIOMARKERS IN PARKINSON DISEASE DIAGNOSTICS AND PROGNOSTICS

Violeta Belickienė[1], Greta Žvinakytė[2,3], Paulina Vaitkienė[1], Daina Skiriutė[2], Aistė Pranckevičienė[4,5], Andrius Radžiūnas[6], Giedrius Steponaitis[2]

[1] *Laboratory of Molecular Neurobiology, Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, Lithuania*

[2] *Laboratory of Molecular Neurooncology, Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, Lithuania*

[3] *Faculty of Medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*

[4] *Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*

[5] *Health Psychology Department, Faculty of Public Health, Lithuanian University of Health Sciences, Lithuania*

[6] *Neurosurgery Department, Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania*

Parkinson's disease (PD) is a progressive neurodegenerative disorder lacking disease modifying therapies, driving interest in extracellular vesicles (EVs) as carriers of regulatory proteins and non coding RNAs. Y RNAs form a substantial yet understudied component of EV cargo and may reflect disease related cellular states, but their cell type specificity, selective EV loading, and clinical relevance in PD remain poorly defined, especially for primate specific Y RNAs requiring human relevant systems.

This study aimed to characterize Y RNA expression in neural cells, determine their selective incorporation into EVs, and assess whether EV associated Y RNA profiles and ratios capture PD related biological and clinical features.

Y RNAs were extracted from four neural stem cell lines, four neural pathology–derived lines, their secreted EVs, and from patient serum derived EVs. RNA was converted to cDNA and quantified by RT qPCR. The study included PD patients, who underwent neurological, cognitive, and quality of life assessment, and

healthy controls. Y RNA expression and ratios were evaluated across cell models and patient EVs to examine group differences, clinical associations, and diagnostic performance.

Y RNAs were differentially regulated across neural cell types, with pathological transformation disrupting their coordinated patterns. Y RNAs were also selectively packaged into EVs, with strong enrichment of RNY4 and relative exclusion of RNY1, indicating active sorting. In serum EVs, PD showed a distinct Y RNA profile: RNY3 related to disease presence, while RNY5 correlated with disease duration, severity, and functional decline. Inter Y RNA ratios provided more informative signals than absolute levels across cell models and patient samples.

Y RNAs emerge as selectively regulated EV cargo with diagnostic and prognostic relevance in PD.

FINANCIAL SUPPORT: This research was funded by The Research Council of Lithuania, grant number S-MIP-23-99.

## S6.1. INTERPERSONAL NEURAL SYNCHRONY IN CLOSE RELATIONSHIPS

Antonia Hamilton

*University College London, United Kingdom*

Prof. Antonia Hamilton's keynote will address the neural mechanisms underlying real-time social interaction, with a particular focus on autism spectrum conditions. Drawing on research from social neuroscience and hyperscanning paradigms, the talk will explore how interpersonal neural synchrony reflects shared representations, action understanding, and social engagement. Prof. Hamilton will discuss how neural coupling differs between typically developing individuals and those on the autism spectrum, and how these differences relate to social skills and interaction quality. The lecture will provide a theoretical framework for understanding synchrony as both a neural and behavioral phenomenon and will set the stage for empirical findings presented in the symposium across different close relationships, including parent–child and romantic dyads.

## S6.2. DETERMINANTS OF INTERPERSONAL NEURAL SYNCHRONY: THE ROLE OF CLOSENESS AND INTERACTION CONTEXT

Alessandro Carollo

*University of Trento, Italy*

Interpersonal neural synchrony is a key characteristic of social interaction, yet findings from hyperscanning studies remain inconsistent due to variability in dyads and task designs. This study examined how relational closeness and levels of social interactivity influence neural synchrony using functional near-infrared spectroscopy (fNIRS) hyperscanning. We recorded brain activity from 142 dyads, including close friends, romantic partners, and mother–child pairs, across three conditions: passive video co-viewing, a structured cooperative task, and unstructured free interaction. Neural synchrony between bilateral inferior frontal gyrus (IFG) and temporoparietal junction (TPJ) was assessed using wavelet transform coherence. Results showed that real interacting dyads exhibited greater synchrony than surrogate pairs, particularly in the right IFG. Mother–child dyads showed lower synchrony compared to adult dyads, suggesting interpersonal closeness effects and developmental influences. At the network level, synchrony was highest during passive co-viewing, followed by cooperative and free interaction, although specific regional patterns peaked during the cooperative task. Overall, social interactivity had a statistically significant but modest effect on neural synchrony, indicating that increased interaction complexity alone may not strongly enhance synchrony in naturalistic settings. These findings contribute to clarifying key factors shaping interpersonal neural synchrony and support more standardized approaches in future hyperscanning research.

## S6.3. EMOTIONAL MODULATION OF CAREGIVER–CHILD NEURAL SYNCHRONY IN AUTISM: INSIGHTS FROM EEG HYPERSCANNING

Joanna Duda-Goławska[1], Weronika Bakun[1], Joanna Beck[3], Agnieszka Pluta[2], Warsaw SYNCC-IN team

[1] *Faculty of Physics, University of Warsaw, Poland*

[2] *Faculty of Psychology, University of Warsaw, Poland*

[3] *SYNCC-IN, University of Warsaw, Poland*

Early caregiver–child interactions are fundamental for the development of emotional regulation and social functioning. Interpersonal neural synchrony (INS) has been proposed as a key mechanism supporting these processes.

In this EEG hyperscanning study we investigated neural synchrony between children with and without autism and their caregivers during joint viewing of short film clips differing in emotional valence (neutral: Peppa Pig; emotional: Brave, The Incredibles). Brain activity was recorded simultaneously from both members of the dyad. Neural synchrony in the theta and alpha bands, extracted from predefined regions of interest (ROIs), was quantified using Multidimensional Recurrence Quantification Analysis (MdRQA), capturing both the strength and temporal structure of shared dynamics (e.g., recurrence rate, determinism, entropy, and vertical line measures).

Preliminary results indicate that emotional context modulates the variability and temporal organisation of neural synchrony. Greater variability across MdRQA measures was observed in the neutral condition, particularly in the autism group, suggesting increased individual differences in dyadic dynamics. In contrast, emotionally salient clips elicited more homogeneous synchrony patterns across dyads. Vertical line measures indicated longer, more continuous shared states in the neutral condition, whereas emotional scenes were associated with shorter, more variable synchrony episodes, consistent with increased co-regulatory processes. Entropy analyses further suggested a more uniform synchrony structure under emotionally engaging conditions. Converging patterns were observed for attentional synchrony.

These findings suggest that emotional salience constrains interpersonal neural dynamics, while low-arousal contexts reveal intrinsic variability in caregiver–child synchrony, particularly in autism. The study highlights the importance of examining the temporal structure of synchrony in naturalistic interactions.

## S6.4. MULTIMODAL ASSESSMENT OF CAREGIVER–CHILD SYNCHRONY: INSIGHTS FROM THE SYNCC-IN PROJECT

Joanna Beck, Jan Łabędź, Maciej Padarz, Mateusz Wawrzyniak, Agnieszka Pluta, Warsaw SYNCC-IN team

*SYNCC-IN, University of Warsaw, Poland*

Understanding caregiver–child synchrony requires integrating behavioral, physiological, and neural levels of analysis. The SYNCC-IN project adopts a multimodal hyperscanning approach to investigate biobehavioral synchrony in mother–child dyads, including both typically developing children and children on the autism spectrum

Data are collected during structured play, joint video viewing, and free conversation. Across these contexts, we simultaneously record EEG, fNIRS, eye-tracking (ET), and heart rate variability (HRV).

This presentation focuses on methodological approaches to analyzing eye-tracking and fNIRS data during joint viewing of short film clips differing in emotional valence. Preliminary results are presented from 30 typically developing mother–child dyads. fNIRS signals were recorded over frontal and temporo-parietal junction (TPJ) regions, while mobile eye-tracking glasses captured gaze alignment and pupil dynamics in both participants.

Results indicate robust interpersonal synchrony across modalities. Higher synchrony was observed during a neutral, low-arousal film, whereas emotionally salient clips were associated with reduced synchrony and increased entropy, suggesting more variable and less stable dyadic dynamics.We further address key methodological challenges in hyperscanning, including how to assess synchrony without a traditional control group and how to construct surrogate comparisons to test research hypotheses.

These findings demonstrate the value of multimodal hyperscanning for studying caregiver–child interaction and provide a practical framework for analyzing interpersonal synchrony in naturalistic contexts.

## S6.5. COMPARING THE SELF AND CO-REGULATION IN LOW AND HIGH STAKES MOMENTS IN AUTISTIC AND NEUROTYPICAL CHILD-CAREGIVER DYADS

Grzegorz Kaliński, Aleksandra Kołakowska, Julia Słowicka, Aleksandra Hamny, Jan Łabędź, Julia Adamczyk, Julia Zaborowska, Alicja Niedźwiecka, Jarosław Żygierewicz, Agnieszka Pluta

[1] *Department of Psychology, University of Warsaw, Warsaw, Poland*

[2] *SYNCC-IN, Horizon 2023*

One of the earliest and most influential interactions occurs between children and caregivers. These exchanges form part of Biobehavioral Synchrony, defined as the matching of behavioral, affective states and biological rhythms between two individuals. Until children develop independent self-regulation (SR), adults provide external regulation, or co-regulation (CR). Over time, regulation progresses from the interpersonal (CR) to the intrapersonal level (SR). This developmental process unfolds within the dyadic child-caregiver context and may be disrupted by autism spectrum disorder (ASD).

The study examined whether this developmental shift from CR to SR is reflected in dyadic motor and physiological synchrony, and whether it is altered in dyads including a child with ASD. We also explored links between interaction patterns and mothers' temperament dimensions.

Interactions were recorded using the SECORE protocol during low-arousal (free play) and high-arousal (e.g., surprise, fear) episodes. Preliminary data include 50 dyads (TD–TD: N=30; TD–ASD: N=20). Using the YOLO model, movement synchrony, spatial distance, and child motor responsiveness (temporal coupling) were automatically coded. Heart rate variability was recorded in both mothers and children.

ASD dyads showed lower movement synchrony and reduced temporal coupling. Mothers of children with ASD followed their child's movements more closely, suggesting a more child-led interaction pattern. Interaction patterns were associated with maternal temperament.

The results suggest that the developmental process progressing from interpersonal co-regulation (CR) to intrapersonal self-regulation (SR) may be altered in ASD. These findings highlight that regulatory processes unfold within the dyadic child-caregiver context and may be shaped by both child characteristics and maternal temperament.

FINANCIAL SUPPORT: Supported by the European Union (Horizon-Widera Europe, grant No. 101159414).

## S7.1. DISTRIBUTED NOCICEPTIVE SYSTEM FRAMEWORK AND ITS IMPLICATIONS FOR UNDERSTANDING PAIN VARIABILITY, HYPERALGESIC AND HYPOALGESIC MODULATIONS OF PAIN

Robert C. Coghill[1,2,3]

[1] *Pediatric Pain Research Center, Cincinnati Children's Hospital, United States of America*

[2] *Division of Behavioral Medicine and Clinical Psychology, Cincinnati Children's Hospital, United States of America*

[3] *Department of Pediatrics, University of Cincinnati College of Medicine, United States of America*

A new overarching conceptual framework for understanding pain, termed the Distributed Nociceptive System (DNS), integrates two neglected concepts – population coding and distributed processing. The central tenet of this framework is that the extraction and utilization of nociceptive information is a process that can be accomplished separately and largely independently by populations of neurons across multiple sites within the central nervous system. As such, processing of nociceptive information can occur in a highly distributed fashion, yielding a system that is very resistant to disruption. The DNS provides a bridge between the basic neuroscience and clinical worlds by providing a mechanistic framework for developing an understanding of the perplexing symptoms of chronic pain. For example, altered receptive field tuning may result in enhanced recruitment of nociceptive neurons and spread of pain. Thus, examining systems supporting spatial tuning may provide important insights into how pain can spread and how this spread can be reversed. Moreover, the widely distributed brain systems that are involved in the construction of the pain experience may require distributed regulation to instantiate either positive or negative changes.

## S7.2. THERMO-NOCICEPTIVE ILLUSIONS IN HUMANS

Alexandra Mitchell
*Aalborg University, Danmark*

Thermo-nociceptive illusions, unusual thermosensory or painful experiences arising from the integration of warm and cold signals, have intrigued researchers for decades. In this talk, I will present our recent work on two such phenomena. The Thermal Grill Illusion (TGI) is characterised by a burning or painful heat sensation when warm and cold stimuli are spatially interleaved on the skin. In contrast, Paradoxical Heat Sensation (PHS) refers to the experience of warmth or heat pain during cooling, induced by temporally alternating warm and cold. I will show that the TGI is driven by the integration of thermal signals within both the spinal cord and the brain. I will argue that contrast is a shared mechanism underlying both illusions, while emphasising marked individual differences in perceptual experience. These findings reveal how thermonociceptive illusions provide a window onto normal thermosensory processing, highlighting the importance of understanding thermosensory integration within the central nervous system.

## S7.3. TEMPORAL CONTRAST ENHANCEMENT IN PAIN

Jakob Pöhlmann

Temporal properties of pain modulation can be investigated using several psychophysical paradigms, one prominent example being offset analgesia. In this phenomenon, a minor decrease in stimulus intensity leads to a disproportionately large reduction in perceived pain, a response thought to be mediated by temporal filtering mechanisms that enhance contrast between small changes in sensory input over time. Similar temporal filtering processes have been documented across multiple sensory domains, raising the possibility that offset analgesia reflects a modality-general mechanism of temporal contrast enhancement rather than a pain-specific phenomenon. In this talk, we will review recent work from our laboratory supporting this view, combining psychophysical and neurophysiological evidence for temporal contrast enhancement in auditory stimulation, and discuss modality-dependent differences in temporal modulation that point to distinct, modality-specific processing patterns.

## S7.4. SPATIAL SUMMATION OF NOCICEPTIVE INPUT AND ITS LIMITS

Wacław M. Adamczyk
*Academy of Physical Education in Katowice, Poland*

Pain is not a simple sum of nociceptive inputs. Instead, spatial interactions between stimulated body regions fundamentally shape pain perception, leading to nonlinear and disproportionate pain reports. In this talk, I will discuss recent psychophysical evidence demonstrating how two core spatial mechanisms—spatial summation and lateral inhibition—interact to modulate pain. I argue that these mechanisms cannot be fully explained by peripheral or spinal processes alone but instead reflect higher-order integration of spatial information. Importantly, I will highlight emerging evidence suggesting that attention mediates spatial pain modulation. This selective amplification or suppression of nociceptive signals depends on task demands and spatial focus. Together, psychophysical findings from my lab and others suggest that spatial pain processing is dynamic and context-dependent, rather than a fixed sensory encoding process. This has important implications for understanding pain modulation in both health and disease.

## S7.5. ESTROGENIC REGULATION OF HUMAN NOCICEPTOR EXCITABILITY: A CELLULAR PLATFORM TO STUDY HORMONAL MECHANISMS IN MIGRAINE

Emilia Goszczyńska[1,2], Angelique Grell[1], Melanie Kuhlmann[1], Leonie Thiele[1], Erhard Wischmeyer[1], Beatrice A. Nossek[1], Oliver Dräger[1]

[1] *Department of Cellular Neurophysiology, Medical School OWL, Bielefeld University, Bielefeld, Germany*

[2] *International Max Planck Research School for Neurosciences, Göttingen, Germany*

Migraine is the most common severe primary headache with a strong sex bias, affecting women 2-3 times more frequently than men. Fluctuations in estrogen levels contribute to migraine susceptibility, yet the cellular mechanisms behind it remain incompletely understood. Human and rodent nociceptors exhibit greater molecular diversity than previously recognized. Therefore, human-derived cellular models are necessary to study pain disorders such as migraine.

This study aimed to differentiate human dermal fibroblasts (HDFa)-derived induced Pluripotent Stem Cells (iPSCs) into peptidergic nociceptive neurons. Secondly, it assessed the influence of phenol red (PR) present in cell culture medium, on neuronal differentiation, in order to consider sources of unintended estrogenic activity in cell cultures. Moreover, we tested the impact of supplemented estrogen on neuronal excitability.

HDFa-derived iPSCs were differentiated into nociceptors in PR-containing medium, PR-free (PR-) medium, or PR- medium supplemented with 17β-estradiol (E2). Neuronal differentiation was assessed by expression of nociceptor markers and electrophysiological characterization. Whole-cell patch-clamp recordings were performed to evaluate action potential firing and membrane currents.

The differentiated cells expressed neuronal markers TUJ1 and ISL1 and peptidergic markers CGRP, SCN11A, TRKA and TRPV1. Cells differentiated in the medium containing PR, known to have affinity for estrogen receptors, exhibited the highest excitability. Supplementation of PR- medium with E2 increased neuronal excitability compared to PR-medium alone.

These findings demonstrate that estrogenic conditions, also unintended, influence nociceptor differentiation and neuronal excitability. This human-derived platform can be utilized as an in vitro model of trigeminal ganglion neurons for further investigation of migraine pathophysiology and sex specific differences.

FINANCIAL SUPPORT: Anschubfond Medizinische Forschung (AMF), Medizinische Fakultät OWL, Universität Bielefeld, Bielefeld, Germany.

## S8.1. INTERPRETABLE MACHINE LEARNING FRAMEWORK REVEALS EVENT-RELATED AND OSCILLATORY EEG SIGNATURES OF EARLY COGNITIVE DECLINE USING A PORTABLE LOW-DENSITY EEG DEVICE AND EMOTIVE STIMULATION PARADIGM

Rosmary Blanco, Luca Gherardini, Jan K. Argasiński

*Sano Centre for Computational Medicine, Kraków, Poland.*

Early detection of dementia remains a clinical challenge and often relies on subjective cognitive assessments. Electroencephalography provides objective biomarkers of neuronal dysfunction, yet clinical adoption is limited by lack of standardisation and limited interpretability of machine learning approaches.

This study aimed to develop and evaluate a framework combining portable EEG devices with explainable artificial intelligence for the early detection of cognitive impairment.

A proof-of-concept study included 24 participants, 15 with Mild Cognitive Impairment and 9 healthy controls, stratified using MMSE and MoCA. EEG was recorded with a 4-channel portable device during a 5-minute passive emotional visual paradigm designed to elicit event-related potentials related to emotion, attention, and memory processing. Extracted features were analysed using tree-based classifiers within a Leave-One-Out Cross-Validation scheme. Robustness and interpretability were evaluated using SHAP analysis, bootstrap validation, and permutation testing.

The Random Forest model achieved an area under the ROC curve of 0.93. At the optimised threshold of 0.56, accuracy reached 87.5 percent, with 86.7 percent sensitivity and 88.9 percent specificity. Bootstrap validation confirmed stability, yielding a mean AUROC of 0.925 with a 95 percent confidence interval from 0.790 to 1.000. Permutation testing indicated statistical significance with p equal to 0.032. SHAP analysis revealed increased N2 latency, increased delta power, and reduced theta and beta activity, indicative of an early sign of cognitive decline, neurodegeneration, early hippocampal dysfunction and loss of synaptic connectivity, respectively.

The framework captures neurophysiological signatures of early cognitive decline using a non-invasive

and scalable approach. It provides objective measures supporting early cognitive assessment and further investigation of dementia-related neural mechanisms.

FINANCIAL SUPPORT: EU Horizon 2020 (857533), FNP (MAB PLUS/2019/13), Polish Ministry of Education and Science (MEiN/2023/DIR/3796).

## S8.2. MACHINE AND DEEP LEARNING APPROACHES FOR AUTOMATED EEG NEUROSCREENING

Maja Marzec, Jarosław Żygierewicz

*Faculty of Physics, University of Warsaw, Warsaw, Poland*

High-dimensional EEG data exhibit high redundancy and low signal-to-noise ratios, complicating automated neuroscreening. This research develops ML-assisted methods to construct reduced, interpretable EEG representations that preserve clinically relevant information.

To integrate dimensionality reduction, connectivity modeling, and generative methods to build interpretable ML-supported systems that enable rapid, objective neuroscreening and serve as clinical decision support tools.

Phase 1 (Bachelor's): Feature-Based Analysis Investigated 2,850-dimensional handcrafted time–frequency features from the ELM19 dataset. Applied PCA and ICA for dimensionality reduction and developed a Selective Activation method to back-project latent components, enabling transparent inspection of original signal drivers. Phase 2 (Master's): Network-Based Analysis Extended work to directed connectivity via multichannel autoregressive models, including Directed Transfer Function (DTF) and frequency-resolved variants. Clustering and aggregation were used to stabilize high-dimensional connectivity vectors. Variational Autoencoders (VAEs) were employed to learn low-dimensional latent spaces, identifying stable connectivity motifs as potential early biomarkers of dysfunction.

In the feature-based phase, applying PCA and ICA to the ELM19 dataset achieved a 90% reduction in dimensionality with only a 2-percentage-point decrease in AUC (0.86 to 0.84) for a Gradient-Boosted Ensemble classifier. The Selective Activation method successfully enabled the identification of specific EEG fragments driving model decisions. Phase 2 is being developed.

With variance-based component selection, dimensionality reduction preserved GBE performance while improving interpretability and generalizability across heterogeneous clinical contexts. Building on this, connectivity-based features introduce network-level representations to further strengthen robust, physiologically meaningful EEG assessment.

FINANCIAL SUPPORT: Google DeepMind AI Master's Scholarship (for the Master's ongoing phase).

## S8.3. PREDICTING RESPONSE INHIBITION: A DEEP LEARNING APPROACH USING PRE-RESPONSE SINGLE-TRIAL EEG DATA

Anna Grabowska[1,2], Paweł Basoń[1,2], Filip Sondej, Magdalena Senderecka[1]

[1] *Jagiellonian University, Centre for Cognitive Science, Kraków, Poland*

[2] *Jagiellonian University, Doctoral School in the Social Sciences, Kraków, Poland*

Response inhibition, the ability to suppress automatic actions in favor of goal-directed behavior, is crucial for self-regulation. It is commonly studied using the stop-signal task (SST), in which frequent go responses must be inhibited when an infrequent stop signal appears. Electroencephalography (EEG) studies have identified neural markers of inhibitory control, yet brain-behavior correlations cannot determine whether observed neural activity reflects inhibition specifically or more general processes that occur in the same time window. Machine-learning approaches may address this limitation by predicting stopping behavior directly from neural signals, but most EEG-based models rely on late post-stimulus activity that may reflect post-response monitoring rather than inhibition itself.

This study used deep neural networks to predict stopping outcomes from EEG activity related to go and stop stimuli while excluding post-response signals.

EEG was recorded from 225 volunteers (113 female, 1 non-binary), aged 18–39, performing an SST. Signals were segmented into go-locked and stop-locked windows and classified using the EEGNet architecture. To compare information across time windows, we trained three models on the following signals: go, stop, and combined go-stop.

Only the go-stop model performed above chance (accuracy=57.8%, AUC=57.7%). Saliency analyses indicated that both go- and stop-related activity within the first 100 ms contributed to classification result. This suggests that successful inhibition depends on interactions between response initiation and stopping pro-

cesses. Importantly, this effect cannot be explained by stop-signal delay (SSD) differences between trial types across participants ($t(224)=.81$, $p=.41$).

Together, these findings show that early perceptual EEG activity predicts stopping success beyond behavioral timing differences, highlighting the role of early interactions between go and stop processes in response inhibition.

FINANCIAL SUPPORT: This study was supported by Sonata Bis grant 2020/38/E/HS6/00490 from the National Science Centre of Poland granted to M.S. A.G. was also supported by the Foundation for Polish Science.

## S8.4. BANDWISE DYNAMIC EEG MICROSTATE CONNECTIVITY AND TRANSITION GRAPH MODELING FOR ALZHEIMER'S DISEASE AND FRONTOTEMPORAL DEMENTIA

Debashis Das Chakladar, Foteini Liwicki, Rajkumar Saini, Esra Sümer Arpak

*Machine Learning Group, Luleå University of Technology, Sweden*

Electroencephalography (EEG) microstates offer a non-invasive window into large-scale brain connectivity and its disruption in neurodegenerative disorders such as Alzheimer's disease (AD) and frontotemporal dementia (FTD).

We aimed to develop a band-wise dynamic microstate and microstate transition framework that characterizes connectivity changes in AD and FTD relative to healthy controls (HC) and links these changes to the cognitive disease stage.

Dynamic microstates were extracted for AD, FTD, and HC across five EEG bands (delta–gamma) using a quantile-based filtering procedure. For each band, brain connectivity-based microstate transition graphs were built by integrating Granger causality and spatial power variations. Local and global graph metrics were quantified using clustering coefficient (CC) and information flow (IF). Between-group differences and associations with AD severity (mild, moderate, severe) were assessed with Mann–Whitney U tests ($p \leq 0.05$).

Compared with HC, both AD and FTD showed significant band-specific alterations in microstate transition graphs. Delta and alpha bands exhibited the strongest CC reductions, whereas beta and gamma bands showed marked IF deficits. In FTD, delta, alpha, and gamma abnormalities were most pronounced. CC and IF changes increased with AD severity.

Band-wise dynamic microstates and their transition graphs capture disease and stage-specific connectivity disturbances, supporting their use as interpretable, non-invasive EEG biomarkers for monitoring neurodegeneration in clinical settings.

FINANCIAL SUPPORT: This research is financially supported by the European Regional Development Fund and the MARTINA-project (no. 20367152).

## S8.5. DESIGNING ARTIFACT-AWARE VIRTUAL REALITY ENVIRONMENTS FOR MOBILE EEG RESEARCH IN HUMAN COGNITION

Julia Caputa[1], Cezary Zając[2], Karina Maciejewska[3]

[1] *University of Silesia in Katowice, Faculty of Humanities, Katowice, Poland*

[2] *University of Silesia in Katowice, College of Interdisciplinary Individual Studies, Katowice, Poland*

[3] *University of Silesia in Katowice, Faculty of Science and Technology, Institute of Biomedical Engineering, Poland*

The integration of virtual reality (VR) with mobile electroencephalography (EEG) enables ecologically valid cognitive research but introduces technical constraints related to rendering latency, frame-dependent timing variability, motion artifacts, and cross-device synchronization. Inadequate control of these factors can compromise event-related potential (ERP) integrity.

The aim of this work was to design a technically robust VR environment architecture optimized for mobile EEG experiments, emphasizing deterministic stimulus scheduling, hardware-level synchronization, and artifact-aware scene construction.

The environment was developed in Unity using a modular control architecture separating stimulus presentation logic, locomotion handling, behavioral input detection, and trigger transmission. Stimuli were preloaded and activated or deactivated at runtime to eliminate instantiation-related latency. Temporal control relied on coroutine-based scheduling executed on the main thread to reduce timing variability. Event markers were transmitted through a USB TTL hardware interface directly to the EEG acquisition system. Marker signals were dispatched at stimulus onset and response detection to ensure temporal correspondence. To stabilize performance, environmental geometry was constrained and lighting was fully baked to reduce rendering load. Head-position tracking was used to monitor vertical displacement during move-

ment, and stimulus flow could be paused to mitigate motion-related artifacts.

System validation demonstrated stable trigger transmission and consistent temporal alignment between VR events and EEG acquisition across stationary and movement conditions.

Careful VR–EEG integration requires explicit control of stimulus timing, rendering performance, and hardware synchronization. The presented architecture provides a technical framework for constructing artifact-aware immersive environments suitable for mobile EEG research in cognitive neuroscience.

FINANCIAL SUPPORT: No external financial support.

## S9.1. PSYCHEDELICS, THE SELF, AND GRIEF

Rebecca Boehme

*Center for Social and Affective Neuroscience, Linköping University, Sweden*

Touch is fundamental to the development and maintenance of a coherent bodily self, emerging from early experiences of both self-touch and caregiver contact. This sense of bodily self remains deeply interwoven with our capacity for social connection and mental health throughout life.

In this talk, I will present findings from my randomized, double-blind, placebo-controlled fMRI study investigating how ketamine, an anesthetic and dissociative substance considered a non-classical psychedelic, alters self-other distinction in the context of affective touch.

Ketamine administration led to dissociative experiences, reduced interoceptive awareness, and diminished neural differentiation between self- and other-touch in a temporoparietal region. These findings illuminate the neural mechanisms by which psychedelic substances might cause ego-dissolution and increased feelings of connectedness.

In addition, I will introduce my new study on the use of psilocybin for prolonged grief disorder, where I will investigate whether psilocybin can support the adaptation to the loss experience by re-establishing the sense of self.

## S9.2. EFFECTS OF CLASSICAL PSYCHEDELICS ON AFFECTIVE STATES AND REWARD PROCESSING IN RATS

Wiktoria Zaniewska

*Nencki Institute of Experimental Biology, Poland*

This work provides insight into the anti-addictive potential of classical psychedelics through preclinical assessment of their effects on affective states in rats.

This study aimed to determine how psychedelics modulate reward processing and to elucidate the contribution of individual differences and social context to these effects.

The utilized protocol combined social isolation periods with group encounters, during which animal behavior was recorded using a behavioral observation system – Live Rat Tracker. Additionally, ultrasonic vocalizations were collected. Psychedelic compounds were administered following isolation periods to assess their ability to modulate positive emotional responses associated with rewarding social contact. By integrating behavioral measures with USV-based readouts and further neurochemistry analysis across both sexes and accounting for individual differences, effects were evaluated.

Across most conditions, classical psychedelics reduced affective responding associated with rewarding group encounters. Changes in positive ultrasonic vocalizations – quantitative indices of emotional valence and arousal – indicated that psychedelics modulate affective responses to rewarding stimuli. These findings are consistent with the idea that reduced affect following psychedelic administration may relate to modulation of dopaminergic reward circuitry, which is central to reward and motivational processing. In line with prior literature, USVs provide a sensitive readout of affective states linked to reward-system activity (including dopaminergic components), supporting their utility as a biomarker of pharmacologically induced shifts in emotion–motivation. There were sex differences observed on some of the psychedelic doses, together with the individual differences in processing across control and experimental conditions.

Psychedelics can shift social reward affect, supporting anti-addictive potential, with sex/individual differences playing an important role.

FINANCIAL SUPPORT: NCN OPUS 26: 2023/51/B/NZ7/02350.

## S9.3. FROM ACUTE NEURAL DYNAMICS TO SUSTAINED RELIEF: CONTRASTING PSILOCYBIN IN HEALTH AND TRD WITH THE SOMATIC CATHARSIS OF MEBUFOTENINE

Marek Nikolic[1,2], Jan Hubený[1], Tomáš Páleníček[1,2], Malin Uthaug[3], Jack Alloca[4], Jan Ramaekers[3], Filip Tyls[1], Jiri Horacek[1,2], Tom Froese[5], Filip Trbusek[1], Isis Koutrouli[1], Falko Cutefurrball[1], Vlastimil Koudelka[1]

[1] *National Institute of Mental Health, Prague, Czech Republic*
[2] *3rd Medical Faculty, Charles University, Prague, Czech Republic*
[3] *University Hospital Maastricht, Netherlands*
[4] *Somnivore*
[5] *Embodied Cognitive Science Unit, Okinawa, Japan*

Psychedelics are recognized for their potential to induce fast-acting, long-lasting changes in cognition and psychological well-being, captivating both psychonauts and therapeutic researchers. This talk presents a triadic view of these compounds, contrasting data from controlled and naturalistic settings.

First, we analyze the acute, time-resolved neural dynamics (EEG) of a single psilocybin dose in healthy subjects, providing high-resolution insight into the compound's immediate neurophysiological footprint. Second, we transition to the clinical sphere, comparing baseline versus post-treatment (Day 1) neural and clinical outcomes of both psilocybin and ketamine in patients with Treatment-Resistant Depression (TRD). This contrast illuminates the distinct sustained effects of these fast-acting agents. Finally, we explore the naturalistic use of Mebufotenine (5-MeO-DMT) via intramuscular administration. We present data on its acute time-resolved dynamics (ECG and EEG), emphasizing how its physiological and experiential profile is markedly different from Psilocybin. Mebufotenine is often associated with intense somatic release: a bodily cathartic experience that is central to spiritual/underground practice yet remains largely unaddressed in clinical trials.

EEG and ECG computational analysis.

We will discuss whether we can distinctly characterise this somatic release within the measured physiological dynamics, underscoring the necessity of integrating naturalistic insights with clinical data.

FINANCIAL SUPPORT: Study was supported by PSYRES, University Maastricht Hospital, The Hefter Institute, the Blue Dot foundation, ERDF-Project Brain dynamics, No. CZ.02.01.01/00/22 008/0004643; Czech Science Foundation (projects 23-07578K), Tiny Blue Dot Foundation, Czech Health Research Council (project NU21-04-00307), the national budget through MEYS, LRI CZECRIN (LM2023049), Long-term Conceptual Development of Research Organization (RVO 00023752), and Specific University Research, Czech Ministry of Education, Youth and Sports (project 260533/SVV/2024), the Charles University research program Cooperatio-Neurosciences, ERDF–Project Brain dynamics, No. CZ.02.01.01/00/ 22 008/0004643, Japan Science and Technology Agency via grant number JPMJPF2205, and PSYRES –Psychedelic Research Foundation. The funding sources were not involved in the study design; in the collection, analysis, and interpretation of data; in the writing of the report.

## S9.4. NO EVIDENCE OF PERSISTENT NEUROPHYSIOLOGICAL REORGANIZATION IN EXPERIENCED USERS OF PSYCHEDELICS

Maja Wójcik[1,2], Paweł Lenartowicz[1,2], Paweł Orłowski[1,3], Stanisław Adamczyk[1,3], Justyna Hobot[1,4], Michał Wierzchoń[1,4], Michał Bola[1]

[1] *Centre for Brain Research, Jagiellonian University, Kraków, Poland*
[2] *Society for Open Science, Warsaw, Poland*
[3] *Doctoral School in the Social Sciences, Jagiellonian University, Kraków, Poland*
[4] *Consciousness lab, Institute of Psychology, Jagiellonian University, Kraków, Poland*

Contemporary neuroscience proposes that acute neurophysiological changes during psychedelic administration translate to lasting reorganization of brain networks. However, critical gaps remain in understanding whether these effects persist in naturalistic users.

Our aim was to investigate whether long-term naturalistic psychedelic users would exhibit neural signatures similar to those observed in acute and post-acute states – in comparison to matched non-users.

We conducted a multi-level EEG investigation comparing experienced psychedelic users (N=57) with matched non-users (N=49). We assessed oscillatory power, neural signal complexity, and source-localized effective connectivity between the Default Mode,

Salience, and Central Executive networks during drug-free resting-state.

Contrary to predictions derived from acute studies, connectivity analyses revealed no significant group differences, while oscillatory power results were inconclusive, yielding no clear evidence for the spectral alterations predicted by acute administration models. Interestingly, users demonstrated reduced rather than increased neural complexity.

These predominantly null findings in ecologically valid samples challenge the assumption that psychedelic use leads to persistent, large-scale neurobiological changes observable in resting-state EEG. The discrepancy between controlled laboratory findings and naturalistic populations suggests a need for methodological pluralism when evaluating the long-term neurophysiological impacts of psychedelics.

FINANCIAL SUPPORT: This project was supported by the National Science Centre, Poland (grant 2020/39/O/HS6/01545) and by the qLIFE and FutureSoc Priority Research Areas under the "Excellence Initiative — Research University" program at Jagiellonian University in Kraków (Competition #6: "Interdisciplinary Collaboration across Medical, Health and Social Sciences").

## S9.5. EMOTIONAL REACTIVITY IN EXPERIENCED USERS OF PSYCHEDELICS

Paweł Orłowski[1,2], Aleksandra Domagalik-Pittner[1], Michał Bola[1]

[1] *Centre for Brain Research, Jagiellonian University, Kraków, Poland*

[2] *Doctoral School in the Social Sciences, Jagiellonian University, Kraków, Poland*

Classic psychedelics profoundly influence emotional states, eliciting intense acute emotional experiences followed by subtle, sustained changes in emotional reactivity lasting up to several weeks. However, it remains unclear whether naturalistic psychedelic use similarly modulates emotional reactivity.

Therefore, this study aimed to investigate behavioral and neural correlates of emotional processing in naturalistic psychedelic users to determine if effects observed in clinical settings translate to real-world use.

We conducted a preregistered, cross-sectional fMRI study comparing experienced psychedelic users (≥10 lifetime experiences; N=33) with a matched group of non-users (N=34) on behavioral and neural responses to emotional facial expressions.

Psychedelic users demonstrated faster and more accurate recognition of angry facial expressions, suggesting reduced interference from threat-related stimuli. Whole-brain fMRI analyses revealed diminished neural responses to anger in limbic and salience network regions, coupled with enhanced responses to happiness in parietal and sensorimotor areas, consistent with prior clinical findings. Additionally, users showed increased precuneus activation in response to fearful facial expressions. Region-of-interest analyses further indicated reduced differentiation of emotional categories in two default mode network regions—the frontal medial cortex and parahippocampal gyrus.

Our study provides a nuanced view of neurofunctional alterations in emotional processing associated with naturalistic psychedelic use, advancing our understanding of its potential long-term effects.

FINANCIAL SUPPORT: This study was funded by a National Science Center Poland grant (grant nr 2020/39/O/HS6/01545).

## S10.1. ROLE OF BRAIN IMMUNITY IN THE REGULATION OF METABOLISM

Agnès Nadjar

*University of Bordeaux, INSERM, Neurocentre Magendie, Bordeaux, France*

Across evolution, female mammals have developed exceptional metabolic flexibility. Hormonal and innate genetic factors enable them to sustain reproduction during periods of food scarcity and efficiently allocate energy while supporting energetically demanding fetuses. This evolutionary advantage gives females a remarkable capacity to maintain energy balance under a wide range of physiological conditions, from handling a calorie-rich meal to withstanding fasting. Central to this regulation is the hypothalamus, which integrates hormonal, neuronal, and nutritional cues. Within this structure, diverse cell populations interact to precisely coordinate whole-body metabolism, including food intake and energy expenditure. Our research aims to uncover the neuroimmune mechanisms that underlie the heightened metabolic resilience seen in females. In my presentation, I will share data demonstrating how microglia and a subpopulation of lymphocytes collaborate to regulate hypothalamus activity and related metabolic function.

## S10.2. THE EFFECT OF MATERNAL HIGH-FAT DIET ON THE PERIPHERAL RETINA OF THE OFFSPRING

Patricija Čepauskytė[1], Gintarė Urbonaitė[1], Neda Ieva Biliūtė[1], Guoda Laurinavičiūtė[2], Urtė Neniškytė[1,3]

[1] *Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania*

[2] *Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Lithuania*

[3] *VU-EMBL Partnership Institute, Life Sciences Center, Vilnius University, Lithuania*

The increased consumption of high-fat diets in modern society is a major contributor to rising obesity rates. Obesity is associated with chronic low-grade systemic inflammation, and maternal high-fat diet (mHFD) has been shown to negatively affect neurodevelopment in offspring. Such inflammatory responses also vary across the female estrous cycle due to hormonal fluctuations. Microglia and Müller cells play key roles in CNS homeostasis, including in the retina. Proteins such as CD68, TSPO, and GFAP are reliable markers of microglial phagocytic activity, metabolic activation, and Müller cell gliosis. Although the effects of high-fat diet on the retina have been studied, little is known about the impact of mHFD on the offspring retina.

This study aims to evaluate the effects of mHFD on microglial morphology, CD68 and TSPO expression, and Müller cell activity in the peripheral retina of offspring, and to assess sex- and estrous cycle–dependent differences.

Firstly, our female C57Bl/6J mice, were fed with either control diet (CD, 10% fat) or high-fat diet (HFD, 60% fat) from weaning through pregnancy and lactation. Offspring were weaned to normal diet. Eyes were fixed, cryosectioned, and immunolabeled for microglia, Müller cells, CD68, and TSPO.

Our results showed that mHFD significantly decreased microglial area in male offspring but increased it in females. While Müller cell area and processes length decreased in females. Alterations observed in CD68 and TSPO induced by mHFD could be due to female hormone changes during female estrous cycle.

These results demonstrate a sex-specific effect of mHFD on offspring retinal microglia and indicate that retinal inflammatory responses may depend on reproductive hormones.

FINANCIAL SUPPORT: This work was supported by the Science Promotion Fund of Vilnius University.

## S10.3. DECODING RESILIENCE: HOW LIPID SIGNALS AND MICROGLIA SHAPE OUTCOMES AFTER ADVERSE CHILDHOOD EXPERIENCES

Weronika Tomaszewska

*Łukasiewicz Research Network- PORT Polish Center for Technology Development, Wrocław, Poland*

Why do some individuals thrive despite adverse childhood experiences (ACE), while others develop neuropsychiatric disorders? Our work explores the biological underpinnings of this resilience–vulnerability spectrum, focusing on peripheral metabolic factors and microglial function. Using human microglia-like cells (HMC3), iPSC-derived microglia, and serum from ethnically diverse ACE cohorts, we discovered that serum from ACE-susceptible individuals disrupts microglial metabolism and synaptoneurosome phagocytosis. Transcriptomic analysis points to altered glucose metabolism as a key driver. Intriguingly, transfection with miR-142-3p—a microRNA differentially expressed in resilient *vs.* susceptible serum—mimicked these effects, identifying it as a potential molecular mediator. This talk will highlight how lipid-mediated signals and microglial responses converge to influence mental health trajectories, opening new avenues for biomarker discovery and targeted interventions.

## S10.4. MICROGLIAL HYPERACTIVATION IN TDP-43 ALS: METABOLIC DRIVERS OF SYNAPTIC PHAGOCYTOSIS

Jan Zakrzewski[1], Ismail Gbadamosi[2], Ali Jawaid[2]

[1] *Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland*

[2] *Translational Neuropsychiatry Research Group (TREND Lab), Łukasiewicz Research Network – PORT Polish Center for Technology Development, Wrocław, Poland*

This presentation will examine the interplay between metabolic and immunological mechanisms driving the progression of amyotrophic lateral sclerosis (ALS) associated with TDP-43 mutations. Emerging evidence indicates that dysregulated microglial activity plays a substantial role in disease progression. The talk will focus on integrated transcriptomic, metabolomic and phenotypic analyses performed in TDP-43 knock-

down murine (BV2) and human (HMC3) microglial cells, modelling the loss of this essential RNA/DNA-binding protein in vivo. It will discuss how alterations in metabolic pathways and gene expression may contribute to an overall hyperphagocytic phenotype, as well as the excessive clearance of both pathogenic and non-pathogenic cargo. Finally, the presentation will highlight current knowledge gaps and consider how new experimental platforms may support deeper mechanistic insight and facilitate the translation of these findings towards therapeutic interventions.

This study aims to demonstrate the mediating role of cellular metabolic pathways in exaggerated synaptic phagocytosis by microglia with TDP-43 loss of function.

TDP-43 was knocked down in murine BV2 and human HMC3 microglial cells using RNA interference. The effects of TDP-43 depletion on microglial metabolism were then examined via glucose uptake and lactate, ATP, and reactive oxygen species (ROS) production assays. Furthermore, live-cell metabolic flux analysis (MFA) of the two cell lines was performed after TDP-43 knockdown under glycolytic and mitochondrial stress. Finally, synaptoneurosome uptake was quantified in cells with TDP-43 knockdown after metabolic rerouting.

Our findings identify TDP-43–dependent microglial metabolic rewiring as a potential driver of pathological synapse loss. The mechanistic links between TDP-43 depletion and a hyperglycolytic, pro-phagocytic microglial phenotype define a novel non-cell autonomous route to neurodegeneration, amenable to attenuation via metabolic rerouting.

FINANCIAL SUPPORT: This project is supported by the EU Joint Programme–Neurodegenerative Disease Research (JPND) (TREMENDOS; UMO-2022/04/Y/NZ5/00122).

## S10.5. DIFFERENCES IN THE METABOLIC PROFILES OF BRAIN LESIONS IN RADIOLOGICALLY ISOLATED SYNDROME AND CLINICALLY DEFINITE MULTIPLE SCLEROSIS REVEALED BY MR SPECTROSCOPY

Maria Sygidus, Marta Agnieszczak, Bartosz Kossowski, Maciej Juryńczyk

*Nencki Institute of Experimental Biology PAS, Laboratory of Brain Imaging, Warsaw, Poland*

Radiologically isolated syndrome (RIS) is characterised by incidental MRI findings suggestive of multiple sclerosis (MS) in individuals without clinical symptoms. Although RIS shares radiological features with MS, the underlying pathobiology remains unclear. Magnetic resonance spectroscopy (MRS) is a non-invasive tool that measures glial and neuronal metabolites in the brain (in vivo), providing insight into the disease process.

This study aimed to investigate metabolic profiles in RIS lesions and normal-appearing white matter using MRS and to compare these profiles with those in MS and healthy controls (HC).

MRS was performed in 30 individuals with RIS, 28 with MS, and 20 HC. In RIS and MS, spectra were acquired both from MS-like T2-hyperintense lesions and normal-appearing white matter (NAWM). In HC, the voxel was placed in cerebral white matter. Metabolite ratios for NAA/(Cr+PCr), NAAG/(Cr+PCr), Ins/(Cr+PCr), GPC+PCh/(Cr+PCr), and Glu+Gln/(Cr+PCr) were analysed.

Lesion analysis revealed significantly increased Ins/(Cr+PCr) in both RIS (Tukey $p=0.002$) and MS ($p<0.001$) compared with HC. Additionally, Glu+Gln/(Cr+PCr) was significantly elevated in MS lesions compared with HC ($p=0.008$). No significant differences were observed in other metabolites in the lesions or between the three groups in normal-appearing white matter.

RIS lesions, similar to lesions from clinically definite MS patients, demonstrate high inositol levels, indicating astroglial activation. However, while MS lesions are characterised by low glutamate levels, RIS lesions do not show impaired glutamate metabolism, likely suggesting earlier or milder pathology. Longitudinal observation will clarify whether inositol in RIS is a predictor for conversion to clinically definite MS.

FINANCIAL SUPPORT: The study is funded and conducted as part of the research component of the National Science Centre 2021/42/E/NZ5/00213: “Predicting prognosis in asymptomatic individuals with multiple sclerosis-like brain lesions using cognitive testing and advanced magnetic resonance imaging techniques”.

## S11.1. ALTERNATIVE ADAPTIVE SOCIAL STRATEGIES EMERGE DURING A SEQUENTIAL SPATIAL MEMORY TASK IN RATS

Krzysztof Maćkiewicz[1,2], Ravindra Sahu[1], Dmytro Tkachenko[3], Witold Żakowski[2], Olga Polniak[2], Johannes Niediek[4], Rafał Czajkowski[5], Ewelina Knapska[6], John P. Aggleton[7], Shane M. O'Mara[8], Maciej M. Jankowski[1]

[1] *BioTechMed Center, Multimedia Systems Department, Faculty of Electronics, Telecommunications and Informatics, Gdańsk University of Technology, Gdańsk, Poland*

[2] *Department of Animal and Human Physiology, Faculty of Biology, University of Gdańsk, Gdańsk, Poland*

[3] *BioTechMed Center, Department of Biomedical Engineering, Faculty of Electronics, Telecommunications and Informatics, Gdańsk University of Technology, Gdańsk, Poland*

[4] *Machine Learning Group, Technische Universität Berlin, Berlin, Germany*

[5] *Laboratory of Spatial Memory, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

[6] *Laboratory of Neurobiology of Emotions, Nencki-EMBL Partnership for Neural Plasticity and Brain Disorders–BRAINCITY, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland*

[7] *School of Psychology, Cardiff University, Cardiff CF10 3AT, United Kingdom*

[8] *Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland*

Understanding how spatial memory guides behavior in social contexts requires experimental paradigms that combine precise task structure with interactive environments.

Here we present an automated, interactive environment (the Switchboard Test) designed to study sequential spatial memory in rats and to examine how such memory is expressed and adapted during social interaction. The system enables training and testing of location- and sequence-specific responses, with adjustable parameters including spatial layout, sequence length, cues, timing, and reward size.

Rats were individually trained to memorize a specific sequence of three button presses and became experts in the task. Expert rats (n=16; two per cage across eight cages) were subsequently tested in pairs with cagemate experts (8 pairs, 9 days), with all possible expert–expert inter-cage pairings (112 pairs, 3 days each), and with naïve rats either from the same cage or from different cages (28 pairs each). Behavioral events were combined with video-based position tracking and individual identification, enabling detailed analysis of joint behavior.

Our apparatus and task enabled rats to deploy ethologically relevant social strategies, including cooperation, temporal segregation, and conflict resolution, which they must learn and refine over repeated interactions to succeed in a social context within an environment with a single reward dispensing area.

Such testing may be useful for identifying individuals with reduced ability to operate in a social context during sequential instrumental tasks, and for studying social hierarchies emerging in an interactive, dynamic environment.

FINANCIAL SUPPORT: National Science Centre, Polonez Bis: 2022/47/P/NZ4/03358 and Gdansk University of Technology, IDUB grant: 1/1/2024/IDUB/I.1B/Pt.

## S11.2. THE NUCLEUS INCERTUS – MODULATORY NODE IN AVERSION-PROCESSING CIRCUITS

Joanna Yadav[1,2], Gniewosz Drwięga[1], Olga Mierniczek[1,2], Martyna Gorkowska-Nosal[1,2], Tomasz Błasiak[1]

[1] *Institute of Zoology and Biomedical Research, Department of Neurophysiology and Chronobiology, Cracow, Poland*

[2] *Jagiellonian University, Doctoral School of Exact and Natural Sciences, Cracow, Poland*

Aversion- and fear-related responses support learning and survival. In humans, impaired regulation of these behaviors contributes to psychiatric and stress-related disorders. The nucleus incertus (NI) is implicated in stress-related processing and projects to key aversion-processing nodes, including the lateral habenula (LHb) and its major efferent target, the rostromedial tegmental nucleus (RMTg).

The aim of the study was to characterize the functional connectivity and anatomical organization of nucleus incertus projections to aversion-processing brain regions, with particular emphasis on lateralization.

To map circuit anatomy, we unilaterally injected (i) retrograde viral tracers expressing fluorescent reporters into the RMTg of adult Sprague-Dawley rats and, in separate animals, (ii) anterograde reporter vectors into the NI. Brain tissue was subsequently examined using fluorescent microscope. To test functional connectivity, we recorded RMTg multi-unit activity in urethane-anesthetized rats during optogenetic stimulation of NI neurons after bilateral RMTg delivery of a retrograde Cre vector and NI delivery of a Cre-dependent opsin.

Retrograde tracing revealed bilateral NI projection to the RMTg. Anterograde tracing showed NI fibers innervating both the RMTg and the LHb, including features of this innervation that have not been described before. No robust modulation of RMTg multi-unit ac-

tivity was detected during NI optogenetic stimulation under urethane anesthesia, suggesting that NI→RMTg influence may be state-dependent and attenuated in an anaesthetized preparation.

Beyond the canonical LHb-RMTg pathway, the NI provides an additional modulatory input to aversion-related circuits. Incorporating NI contributions may refine circuit-level models of aversion and stress processing.

FINANCIAL SUPPORT: Preludium 21 2022/45/N/NZ4/03171.

## S11.3. RECRUITMENT OF THE INTERPEDUNCULAR NUCLEUS–VENTRAL HIPPOCAMPUS PATHWAY DURING SOCIAL STRESS

Sylwia Drabik[1,2], Aleksandra Trenk[1], Anna Guguła[1], Gabriela Stopka[1,2], Gabriela Czerniak[1], Aya Dridi[2,3], Patrycja Malewska[1], Angelika Kaleta[1], Patryk Sambak[1,2], Anna Błasiak[1]

*[1] Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

*[2] Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

*[3] Department of Cell Biology and Imaging, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

Social stress elevates brain nerve growth factor (NGF) levels, reshaping the activity of stress-sensitive circuits. The midbrain interpeduncular nucleus (IPN), a regulator of anxiety and social behaviour shows a robust expression of the NGF receptor TrkA and sends dense projections to the ventral hippocampus (vHPC); a key hub for social and anxiety-related signalling and an important NGF source.

Despite this anatomical link, its behavioural relevance and mechanistic role of the IPN–vHPC pathway remain unclear. Accordingly, we sought to define the electrophysiological properties and possible functional role of IPN neurons innervating the vHPC.

Molecular characterization of IPN neurons was performed using RNAscope in situ hybridization. Viral based tract tracing was employed to map IPN-vHPC innervation. Electrophysiological whole cell patch clamp and multielectrode array recordings were used to verify IPN neurons' sensitivity to NGF. To examine behavioral engagement of the circuit, resident-intruder paradigm followed up by assessment of neuronal activation was carried out.

RNAscope analysis revealed that TrkA-expressing IPN neurons are mainly located in rostral, intermediate, and lateral subnuclei, aligning with viral tracing data identifying vHPC-projecting cells in these areas. Patch-clamp recordings from anterior IPN slices showed no detectable response to NGF. Electrophysiological recordings with optogenetic tagging of IPN neurons innervating vHPC, revealed a small subset of identified neurons responding to NGF with inward currents. Multielectrode array recordings showed heterogeneous effects of NGF, of IPN neurons. Social stress exposure in the resident-intruder test resulted in the increased c-Fos expression in IPN cells.

Our findings reveal that the IPN-vHPC circuit is enriched in TrkA-expressing subregions, exhibits selective and heterogeneous sensitivity to NGF, and is engaged during social stress, supporting its role in modulating stress-related behaviors.

FINANCIAL SUPPORT: Funding: National Science Centre, Poland (UMO 2021/41/N/NZ4/04499; UMO-2023/49/B/NZ4/01885.

## S11.4. NEURAL MECHANISMS OF SPATIAL CHOICE: DCA1 PLASTICITY, CUE PROCESSING & BEHAVIORAL VARIABILITY

Julia Sepielak-Świderska[1], Lali Kruashvilli[1], Galip Yiğit Ünlü[1], Małgorzata Grochowicz[1], Błażej Ruszczycki[2], Kasia Radwańska[1]

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

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

Hippocampal synaptic plasticity, in particular long-term potentiation (LTP), has long been considered a central mechanism underlying spatial memory. However, recent evidence from studies in genetically modified mice has challenged this concept, proposing instead that hippocampal plasticity may play a role in spatial decision-making (Bannerman et al., 2012).

To examine the molecular and behavioural determinants of spatial choice, we designed a fully automated behavioural platform that enables continuous tracking of mouse locomotion and navigation. The system comprises three interconnected corridors outfitted with video monitoring, visual and auditory cue modules, automated doors, and liquid reward delivery, allowing animals to explore freely while acquiring rewards.

Using this paradigm, we assessed spatial decision-making in male and female C57BL/6 mice and observed no sex differences in overall performance. We systematically evaluated the contributions of motivational state, cue modality, reward distance, and dorsal CA1 (dCA1) synaptic plasticity. Plasticity was disrupted via lentiviral shRNA-mediated knockdown of the synaptic scaffold protein PSD-95.

Food restriction enhanced motivation, learning efficiency, and choice dynamics, while closer reward placement accelerated acquisition. Cue manipulations in males showed that removal of visual cues impaired performance. PSD-95-dependent dCA1 plasticity emerged as a key regulator of attentional engagement and decision-making strategies. Notably, animals could be segregated into high-, intermediate-, and low-performing subgroups, indicating inherent differences in decision strategies. To further investigate the neural correlates underlying performance variability between groups, whole-brain c-Fos immunostaining across serial sections was performed.

Collectively, these results elucidate how hippocampal plasticity, environmental information, and individual variability interact to shape spatial decision-making.

FINANCIAL SUPPORT: National Science Centre 2020/38/A/NZ4/00483 to K.Radwańska.

## S11.5. LATERALISED NEURONAL NETWORKS OF REWARD SEEKING ACROSS MOUSE LIFESPAN

Monika Puchalska[1], G.Yigit Unlu[1], Marcin Barański[2], Marzena Stefaniuk[2], Kasia Radwańska[1]

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

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

Reward seeking behaviors are supported by distributed neuronal networks that functional organisation evolves throughout aging. Understanding age-dependent differences in neuronal basis of behavior is crucial for elucidating the mechanisms underlying cognitive decline and age-related neurological disorders.

The aim of this study is to investigate how lateralised neural circuits underlying reward seeking are shaped across mouse lifespan.

This study investigates neuronal networks of social learning in mice across different age groups using IntelliCage automated behavioral system, whole brain iDISCO clearing coupled with c-Fos immunostaining and chemogenetic manipulations.

We observed significant variations in behavior between young (3-5 months old) and old (>18 months old) mice, particularly in exploratory activity, social behaviors, learning and memory tasks. Further, iDISCO analysis revealed age-related differences in the activation patterns of brain regions associated with olfactory and cognitive functions. In particular, we observed strong lateralization of old mice brain activity in hippocampal and amygdalar areas. This phenomenon was much less pronounced in young animals. Chemogenetic manipulations confirmed specific engagement of the left, but not right, medial nucleus of the amygdala in social preference and learning again only in old, but not young, mice.

Our study demonstrates that spontaneous behavioral strategies employed by mice in order to find reward change as animals age, and this process is accompanied by evolving brain network activity. In particular, we show for the first time that mouse brain activity is strongly lateralised and this process progresses over time.

FINANCIAL SUPPORT: This work is supported by the National Science Centre MAESTRO Grant (2020/38/A/NZ4/00483) to K.R.

## S11.6. FROM SUPERIOR COLLICULUS TO STRIATUM: A LATERALIZED PATHWAY LINKING VISUAL SPACE TO DOPAMINE AND ORIENTING

Martyna Marzec[1,2], Karolina Nowalińska[1], Dominika Moskal[1], Alicja Stankiewicz[1], Tomasz Błasiak[1]

*[1] Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Cracow, Poland*

*[2] Jagiellonian University, Doctoral School of Exact and Natural Sciences, Cracow, Poland*

Midbrain dopaminergic (DA) neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) shape action selection through dopamine release in the striatum. Transient hemispheric imbalances in dopaminergic signaling can bias directional behaviours. The superior colliculus (SC) conveys spatially organized multisensory information to VTA/SNc, but the extent to which this input supports lateralized dopaminergic output and biases orienting movements remains unclear.

We aimed to define the anatomical organization and functional lateralization of the SC→VTA/SNc→striatum pathway in rats, and to determine how unilateral visual stimulation differentially modulates

DA neurons activity and hemispheric dopamine dynamics in rat.

Anatomical connectivity was examined using viral tract-tracing. In vivo extracellular single-unit recordings from VTA/SNc DA neurons were performed during pharmacological disinhibition of the SC combined with uniocular light stimulation. Striatal DA dynamics were quantified using fiber photometry. The behavioural relevance of this circuit was assessed using pathway-specific optogenetic stimulation during custom-made orienting test.

Tract-tracing revealed pronounced lateralization and topographic organization within the circuit, with SNc DA neurons receiving SC input preferentially innervating the ipsilateral dorsal striatum. Uniocular stimulation predominantly evoked excitatory responses in VTA/SNc DA neurons. Although firing rates didn't show a consistent hemispheric bias, striatal dopamine release was significantly higher contralateral to the stimulated eye. Optogenetic activation of the pathway induced bias in animals' orienting behaviour.

These results indicate that lateralized visual input differentially modulates dopamine signals through anatomically asymmetric SC to midbrain dopaminergic circuit. This pathway may provide a mechanism by which spatial sensory information may bias action selection.

## S12.1. VISUAL FIELD ASYMMETRIES IN TEMPORAL OBJECT PERCEPTION – BEHAVIORAL AND NEUROPHYSIOLOGICAL CORRELATES

Julia Papiernik-Kłodzińska[1,2,3], Marek Binder[1], Renate Rutiku[1]

[1] *C-lab, Institute of Psychology, Jagiellonian University, Kraków, Poland*

[2] *Centre for Brain Research, Jagiellonian University, Kraków, Poland*

[3] *Doctoral School in the Social Sciences, Jagiellonian University, Kraków, Poland*

It is well established that visual perception varies across the visual field. Performance is better along the horizontal than the vertical meridian of the visual field (horizontal-vertical asymmetry, HVA), and along the lower than the upper half of the vertical meridian of the visual field (vertical meridian anisotropy, VMA). These phenomena have been demonstrated in various tasks. However, it is not apparent to what extent they influence temporal object perception.

The study aimed to determine whether temporal object perception is modulated by visual field location, and to test whether HVA and VMA extend to subjective temporal perception.

25 healthy participants took part in the study. We used a custom experimental paradigm combining figure-ground modulation with steady-state visual potential (SSVEP) stimulation to assess the disparities between subjective perception and objective neural markers of stimulus processing. Pop-out figures emerged from a background of rotating line segments flickering randomly at 6 and 15 Hz. The figures appeared in one of eight locations across the visual field and flickered at 6 Hz. The objective stimulus duration consisted of either 5, 6, or 7 cycles. Participants counted the perceived number of flickers. Neurophysiological responses were recorded using a 64-channel BioSemi EEG system.

Participants systematically underestimated stimulus duration, reporting on average 4.56 figure flickers per trial. Location significantly affected behavioral responses, revealing HVA but no VMA. Overall accuracy was low (0.16). ERP amplitude varied as a function of stimulus polar angle. Effects of stimulus duration were less consistent and will require further investigation. SSVEP responses at 6 Hz were elevated during figure relative to baseline and similarly varied by location. Ongoing analyses will additionally examine the time-frequency dynamics across stimulus durations and locations.

Visual field location modulated both behavioral and neural responses, confirming that temporal object perception is not uniform across the visual field.

FINANCIAL SUPPORT: This work was supported by the National Science Centre, Poland (grant number 2021/42/E/HS6/00425).

## S12.2. IT'S COMPLICATED: OPTIMAL VISUAL FEATURES FOR MAXIMIZING SSVEP RESPONSES ARE FREQUENCY-DEPENDENT

Sofiia Honcharova[1,2], Renate Rutiku[2]

[1] *Doctoral School in the Social Sciences, Jagiellonian University, Kraków, Poland*
[2] *Institute of Psychology, Jagiellonian University, Kraków, Poland*

Steady-state visual evoked potentials (SSVEPs) are an increasingly popular methodology in vision research and beyond. Yet, a comprehensive understanding of what drives the response magnitude remains limited. To date, systematic comparisons have been largely restricted to square versus sine waves, leaving a gap in practical guidelines for optimising SSVEP paradigms.

This study aimed to (I) systematically map the effects of multiple stimulus parameters on SSVEP strength across frequencies; (II) better understand the neural basis of SSVEP generation.

Twenty-nine participants completed a within-subject experiment crossing three frequencies (6, 15 & 40 Hz), three flicker types (on/off square, sine and 2-frame "tick" waves), two contrast levels (high contrast *vs.* isoluminant) and two visual complexity levels (low: uniform squares *vs.* high: dynamic stochastic "sprinkles" pattern). EEG was recorded alongside trial-by-trial subjective flicker visibility ratings.

Apart from frequency, contrast had the largest influence on SSVEP power (high contrast > isoluminant), while complexity emerged as the second most impactful factor. Flicker type had the smallest, mostly negligible effect. This pattern was also mirrored in the subjective flicker strength ratings. Critically, the effects of complexity reversed across frequencies: while low-complexity stimuli elicited stronger SSVEPs at 15 & 40 Hz, high-complexity stimuli yielded stronger SSVEPs at 6 Hz. Topographical analysis further supported the dissimilarity of SSVEP responses across frequencies.

SSVEP generation appears to operate differently across frequency ranges. Stimulus complexity emerged as a previously overlooked yet significant determinant of SSVEP magnitude. These results challenge assumptions about SSVEP uniformity and carry direct practical implications for paradigm design. Additionally, this work raises broader questions about the distinct neural processes engaged at different stimulation frequencies.

FINANCIAL SUPPORT: This work was supported by research grant nr 2021/42/E/HS6/00425, awarded by the National Science Center of Poland.

## S12.3. POSTERIOR THETA REFLECTS SUPPRESSION OF COMPETITIVE S-R LINKS

Rob van der Lubbe[1,2], Alexandru Serban[2], Bartłomiej Panek[3], Dariusz Asanowicz[3]

[1] *Faculty of Physics, Adam Mickiewicz University, Poznań, Poland*
[2] *Faculty of Behavior, Management, and Social Sciences, University of Twente, Enschede, The Netherlands*
[3] *Institute of Psychology, Jagiellonian University, Kraków, Poland*

Recent studies employing the electroencephalogram (EEG) revealed that posterior theta after the presentation of arrays in the Eriksen task is sensitive to cognitive conflict. This posterior effect precedes effects on theta power over midfrontal areas, suggesting that it is an earlier sign of conflict detection. A few studies observed that this effect is largest when the flankers signal the alternative response.

Two experiments were performed to further examine these effects on posterior theta.

In the first experiment, we aimed at a conceptual replication with another version of an Eriksen task. The central target (C or S) required a left, or a right (H or K) response, and arrays were either congruent (e.g., CCC or KKK), incongruent but response compatible, (e.g., CSC or KHK), or incongruent and response incompatible (e.g., KCK or SKS). In a second experiment, we examined sequential effects in a standard Eriksen task with response compatible (e.g., SSS) and response incompatible arrays (e.g., HSH).

Experiment 1 replicated the largest increase in posterior theta for incongruent and incompatible arrays. Experiment 2 revealed largest posterior theta in the case of a repetition of incompatible arrays with response repetition (I-I-R), smallest posterior theta in the case of a repetition of identical compatible arrays (C-C-R), but also relatively low theta when a compatible array was followed by an incompatible array with a response repetition (C-I-R). However, responses for the latter sequence were slowest and the least accurate.

These results may indicate that multiple mechanisms are involved resolving conflicts in the Eriksen task. One mechanism, reflected in posterior theta, may reflect suppression of competitive S-R links at an early sensory level, preventing the propagation of interfering information along the dorsal pathway, while a second more central mechanism intervenes in the case of a low level of early suppression.

FINANCIAL SUPPORT: This work was supported by one grant from the National Science Center (Narodowe Centrum Nauki) of Poland awarded to R.L. (2019/33/B/HS6/00096), and one awarded to D.A. (2022/45/B/HS6/01107).

## S12.4. BEYOND VALENCE: VISUAL FEEDBACK TYPE MODULATES NEURAL CORRELATES OF FEEDBACK PROCESSING

Paweł Basoń[1,2], Filip Sondej[2], Magdalena Senderecka[2]

[1] *Doctoral School in the Social Sciences, Jagiellonian University, Kraków, Poland*
[2] *Centre for Cognitive Science, Jagiellonian University, Kraków, Poland*

Performance monitoring, particularly feedback processing, plays an important role in multiple areas, such as learning or decision-making. Depending on specific objectives, researchers use various types of feedback stimuli, which can make the interpretation and comparison of results across studies more challenging. To date, relatively few studies have systematically examined how qualitative differences in feedback format influence the neural correlates of feedback processing. Consequently, discrepancies observed between studies may not solely reflect theoretical manipulations (e.g., reward *vs.* punishment) but may instead result from low-level perceptual features (e.g., visual complexity, salience) or higher-level representational properties (e.g., symbolic *vs.* social content).

The aim of the study was to examine how the type of visual feedback influences neural correlates of performance monitoring.

103 participants (73 female) performed a Time Estimation Task while EEG data were recorded. Participants received performance feedback presented in one of six different visual formats: color, text, points, face, emoticon, or a pictogram. The analysis focused primarily on Feedback-Related Negativity (FRN) as a key neural marker of feedback processing, while not being limited exclusively to this component.

The analysis revealed a significant main effect of feedback type, $F(5, 115.98)=324.62$, $p<.001$. Additionally, there was a significant valence × type interaction, $F(10, 115.98)=7.20$, $p<.001$, demonstrating that the effect of valence on FRN amplitude depended on feedback type. Additionally, a series of *post hoc* analyses was conducted to identify which stimulus pairs within each valence condition exhibited the most pronounced differences.

These results support the view that early feedback processing reflects an integration of evaluative and representational stimulus features rather than a purely valence-driven response.

FINANCIAL SUPPORT: This study was funded by the Excellence Initiative program for PhD students Research Support Module 2025 granted to P.B. & by a grant from the Faculty of Philosophy under the Strategic Programme Excellence Initiative at Jagiellonian University granted to M.S. This study was also supported by Sonata Bis grant 2020/38/E/HS6/00490 from the National Science Centre of Poland.

## S12.5. PERFORMANCE MONITORING AND DECISION FORMATION UNDER UNCERTAINTY: ERP EVIDENCE FROM A SPEEDED RAVEN'S TASK

Julia Klimecka[1,2], Filip Sondej[2], Adam Chuderski[2], Magdalena Senderecka[2]

[1] *Doctoral School in the Social Sciences, Jagiellonian University, Kraków, Poland*
[2] *Centre for Cognitive Science, Jagiellonian University, Kraków, Poland*

Performance monitoring has primarily been studied in simple speeded paradigms with unambiguous outcomes, where the error-related negativity (ERN) reliably differentiates erroneous from correct responses. It remains unclear whether this marker generalizes to complex reasoning tasks in which decisions unfold over extended intervals and remain ambiguous at response execution.

The primary aim of this study was to test whether classical response-locked components, such as ERN/correct-related negativity (CRN) and error/correct positivity (Pe/Pc), differentiate correct and incorrect responses in a speeded reasoning task. The secondary aim was to characterize neural indices of decision formation by examining the centroparietal positivity (CPP) as a marker of evidence accumulation and stimulus-locked components related to matrix processing (N1, P3, LPP).

EEG and behavioral data were collected from 97 participants (49F; 48M) aged 18 to 35 years ($M=23.76$, $SD=4.03$) while they completed a modified, speeded version of Raven's Standard Progressive Matrices. ERN/CRN and Pe/Pc were first examined using a standard pre-response baseline and, exploratorily, with an ear-

lier baseline to account for possible pre-response error-related activity.

Using the conventional baseline, the ERN and Pe amplitudes did not significantly differentiate errors from correct responses. Baseline adjustment yielded only a marginal correctness effect. In contrast, the CPP was significantly larger for correct than error trials (p=.009), whereas the LPP (p=.017) and N1 (p=.011) were enhanced for errors. The P3 did not mirror CPP effects, supporting a dissociation between these components, which are often interpreted as reflecting the same underlying decision process.

These findings suggest that classical response-locked error signals may be attenuated in complex reasoning under uncertainty, whereas centroparietal decision dynamics remain sensitive to decisional outcomes.

FINANCIAL SUPPORT: This work was supported by a Sonata Bis grant 2020/38/E/HS6/00490 from the National Science Centre of Poland to MS. Data collection was funded by a Sonata Bis grant 2017/26/E/HS6/00595 from the National Science Centre of Poland to AC.

## S13.1. PSYCHEDELIC THERAPY – PROMISE, NEUROPSYCHOLOGICAL MECHANISMS AND POSSIBLE ROADMAP TO CLINICAL PRACTICE

David Erritzoe

*IMPERIAL COLLEGE – Centre for Psychedelic Research & CIPPRes Clinic, Division of Psychiatry, Dpt of Brain Sciences*

In this talk, Dr David Erritzoe will outline the field, from the psychological and neurobiological role of psychedelics in increasing flexibility in "stuck" brain networks to key findings from recent clinical trials. He will then examine the practical realities of implementation, exploring how healthcare systems can prepare for the emerging wave of psychedelic therapies. Using ketamine service pilots as an example, David will show how collaboration between public health systems and new treatments can provide a balanced, scalable path forward — expanding access while strengthening the evidence base within existing care structures.

## S13.2. INTEGRATED BEHAVIORAL AND MOLECULAR ANALYSIS OF PSILOCYBIN EFFECTS IN C57BL/6 WT MICE

Jakub Mlost, Adam Wojtas, Shaima Islam, Yamen Levain, Zhaokun Wang, Iskra Pollak Dorocic

*Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Sweden*

Psilocybin, a serotonergic psychedelic, induces marked behavioral and molecular changes, yet how altered behavior relates to the underlying transcriptome remains poorly understood.

This study aimed to establish a direct relationship between psilocybin-induced behavioral dynamics and region-specific transcriptional responses by combining high-resolution behavioral analysis with spatial transcriptomics (ST) in individual animals.

C57BL/6 mice received 0.125, 1, or 3 mg/kg psilocybin (i.p.) and underwent 60-minute Open Field Tests (OFT), followed by brain collection for 10x Genomics Visium ST. Behavioral videos were processed using DeepLabCut. Classical OFT metrics were quantified with DLCanalyzer, while distinct behavioral motifs were identified using the B-SOiD. ST data were analyzed in Seurat, and Canonical Correlation Analysis was used to link gene expression with behavior.

OFT revealed dose- and time-dependent effects: 1 mg/kg psilocybin induced early hyperlocomotion, whereas 3 mg/kg produced delayed hypolocomotion with increased peripheral occupancy, consistent with anxiogenic-like behavior. B-SOiD identified over 10 behavioral motifs, with sniffing and rearing selectively modulated in a dose-dependent manner. ST resolved molecular clusters corresponding to different neuroanatomical structures. We identified 1,397 cluster-specific psilocybin-induced differentially expressed genes (DEGs), with DEGs abundance positively correlated with regional expression of Htr2a. Canonical correlation analysis uncovered the underlying transcriptomic signature that significantly influenced behavioral patterns, revealing potential molecular substrates underlying psychedelic-induced behavioral states.

Psilocybin-induced changes in behavioral changes are strongly associated with underlying molecular patterns/ This integrative approach provides a framework for mechanistically linking the behavioral and transcriptional dimensions of psychoactive compounds.

FINANCIAL SUPPORT: This study was supported by SciLifeLab, Wenner-Gren Foundation and the Swedish Research Council grants to I.P.D. and the SciLifeLab RED Postdoctoral Fellowship to J.M.

## S13.3. A STRIOSOMAL MECHANISM FOR PSYCHEDELIC-INDUCED SUPPRESSION OF SELECTIVE COMPULSIVE-LIKE BEHAVIOURS

Vasiliki Skara, Ioannis Mantas, Alessandro Contestabile, Konstantinos Meletis
*Karolinska Institutet, Neuroscience Department, Biomedium, Solna, Sweden*

Psychedelic compounds have recently attracted considerable attention due to their rapid and long-lasting antidepressant effects. Despite extensive characterization of their pharmacological properties, the circuit-level mechanisms underlying their therapeutic actions remain poorly understood. The striatum has emerged as a key region in the pathophysiology of mood and compulsive disorders. In particular, the striosome compartment, characterized by dense mu-opioid receptor (Oprm1) expression, has been implicated in psychomotor and affective dysfunction.

Here, we report an enrichment of the psychedelic receptor 5-HT2A (5HT2aR) within striosomes, the primary target of the 5-HT2A agonist 2,5-dimethoxy-4-iodoamphetamine (DOI). DOI administration resulted in reduced c-FOS expression in the striatum. Using calcium imaging of Oprm1+ neurons, we show that DOI desynchronizes neuronal activity at the population level and alters activity patterns associated with specific behavioral motifs. Furthermore, chronic silencing or activation of striosomal neurons produces selective changes in compulsive-like behavioral sequences following DOI administration compared to control animals.

Together, our findings identify a striosome-specific circuit mechanism through which psychedelic signaling may modulate pathological behavioral patterns, providing new insight into the neural substrates underlying psychedelic-mediated therapeutic effects.

FINANCIAL SUPPORT: Karolinska Institutet Doctoral funding (KID).

## S13.4. DISTINCT BEHAVIORAL AND ULTRASONIC VOCALIZATION PROFILES OF DMT, PSILOCYBIN, AND LSD IN RATS: A TRANSLATIONAL WINDOW INTO PSYCHEDELIC ACTION

Wiktoria Zaniewska
*Nencki Institute of Experimental Biology PAS, Warsaw, Poland*

Classical psychedelics share serotonergic targets yet may engage behaviors through partially non-overlapping mechanisms.

This study directly compared the dose-dependent behavioral and ultrasonic vocalization (USV) profiles of DMT, psilocybin, and LSD, treating each compound as producing a unique behavioral-vocal signature rather than a uniform psychedelic effect.

Rats underwent a social isolation-encounter protocol with simultaneous behavioral and USV recording. Critically, USV production in rats is neurobiologically grounded: 50-kHz calls are linked to dopaminergic activation of the nucleus accumbens and reflect positive motivational states, while 22-kHz calls are associated with aversive arousal via distinct limbic circuits.

This mechanistic framework allowed us to move beyond treating USVs merely as emotional proxies. Instead, their acoustic structure, call-type ratios, and temporal dynamics served as compound-specific readouts of underlying neurochemical state. DMT, psilocybin, and LSD each produced distinguishable dose-dependent patterns in both behavior and USV emission, differing not only in magnitude but in the qualitative character of behavioral responding.

These divergent profiles carry direct translational relevance: if each compound modulates dopaminergic-serotonergic crosstalk differently, USV signatures may serve as preclinical predictors of differential therapeutic outcomes in humans. Grounding behavioral observation in known molecular mechanisms of vocalization offers a sensitive, non-invasive framework for mapping psychedelic action.

FINANCIAL SUPPORT: Grant OPUS 26: Investigating the neurobiological mechanisms of the anti-addictive effects of psychedelics, 2023/51/B/NZ7/02350.

## S13.5. THE ROLE OF 5-HT2A RECEPTOR IN SLEEP ARCHITECTURE AND MEMORY CONSOLIDATION IN AN ANIMAL MODEL

Kristýna Aleksić[1,2], Lucie Ladislavová[1], Klára Šíchová[1], Eva Lhotková[1], Čestmír Vejmola[1], Tomáš Páleníček[1,2]

[1] *National Institute of Mental Health, Psychedelics Research Centre, Klecany, Czech Republic*

[2] *Charles University, Third Faculty of Medicine, Prague, Czech Republic*

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 is a neuroplastic serotonergic psychedelic currently explored for its therapeutic potential in psychiatric disorders.

To examine the effects of psilocybin, a non selective serotonin receptor agonist, MDL100907, a selective 5-HT2A receptor antagonist, and their combination on sleep parameters and hippocampus dependent memory in adult male Long Evans rats, with and without acute sleep deprivation.

Animals were implanted with EEG/EMG telemetry devices to enable continuous sleep monitoring. Treatments were administered sc., and animals were either left undisturbed or sleep-deprived for 8 hours during the light phase. A within subject design was used. Biotelemetric data were recorded for 24 hours following treatment. Memory consolidation was assessed using the object place recognition task and the Morris water maze. Testing occurred after the sleep recording period to evaluate delayed effects.

Psilocybin and MDL100907 did not significantly affect NREM sleep but produced marked alterations in REM sleep. REM onset latency increased in all drug treated groups compared to controls, and REM duration was reduced. Psilocybin also increased wakefulness during the light phase. Sleep deprivation reduced both NREM and REM sleep, confirming the effectiveness of the protocol. No significant effects were found on memory performance in either behavioral task, and no synergistic or protective effects were observed in the combined treatment group.

Modulation of the 5-HT2A receptor selectively disrupted REM sleep architecture without affecting NREM sleep or behavioral measures of memory consolidation. These findings suggest that serotonergic systems may regulate sleep phases independently of short term cognitive outcomes and contribute to understanding sleep cognition interactions in the context of serotonergic modulation.

FINANCIAL SUPPORT: This work was supported by grant from Czech Health Research Council (project NU21-04-00307 and NW24-04-00413), Czech Science Foundation (20-25349S), GAČR (Ratcon) – EEG/ECoG based functional connectivity neuroimaging in the rat – optimisation, standardisation and translational leap in neuropsychopharmacology, GAČR no.: 23-07578K, Ministry of the Interior of the Czech Republic (project VK01010212), PsyPal project from Horizon Europe (grant no. 101137378, HORIZON-HLTH-2023-DISEASE-03-01) and Charles University research program Cooperatio-Neurosciences and private funds obtained via PSYRES, Psychedelic Research Foundation (https://psyresfoundation.eu).

## S14.1. AXONAL OXYTOCIN SIGNALING IN RAT AND HUMAN BRAIN

Valery Grinevich[1,2]

[1] *Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health, Heidelberg University, Germany*

[2] *International Joint Laboratory for Translational Research on Neuromodulation, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China*

Over the past decade, major advances have deepened our understanding of the oxytocin (OT) system, yet a central question remains: how can a single neuropeptide mediate such diverse actions in the brain? I propose that this diversity arises from distinct types of OT neurons, their widespread axonal projections, and the variety of OT-responsive cell populations across brain regions. In this talk, I will focus on axonal OT projections to the medial prefrontal cortex (mPFC) in rats, demonstrating OT action on local interneurons and its profound facilitation of social interactions. I will also present complementary findings from human stem cell-derived OT neurons, which extend axons toward cortical assembloids and, when integrated into the rat hypothalamus, form functional connections with multiple brain regions and the pituitary. Together, these results reveal conserved principles of axonal OT signaling across species, shedding light on the mechanisms underlying the peptide's diverse functions in the mammalian brain.

FINANCIAL SUPPORT: The Synergy European Research Council (ERC) grant "OxytocINspace" 101071777, SFB Consortium 1158-3, DFG grant 533293533, German-Israeli Project cooperation (DIP) GR3619-1, and ERANET-Neuron grant GR 3619/25-1.

## S14.2. MAPPING THE MOUSE PITUITARY GLAND IN SPACE: A TRANSCRIPTOMIC VIEW OF THE POSTERIOR LOBE

Ewelina Kałużna[1], Andranik Ivanov[2], Julian Zacharjasz[1], Dieter Beule[2], Monika Piwecka[1]

[1] *Institute of Bioorganic Chemistry, Polish Academy of Sciences, Department of Non-coding RNAs, Poznań, Poland*

[2] *Berlin Institute of Health at Charité, Core Unit Bioinformatics, Berlin, Germany Berlin, Germany*

The pituitary gland is a central neuroendocrine interface, integrating hypothalamic inputs to regulate systemic physiology, including growth, metabolism, reproduction, and stress responses. Despite its fundamental role, the spatial organization and local transcriptomic landscape of the adult mouse pituitary gland remain poorly understood.

In this study, we aimed to generate a spatially resolved transcriptomic map of the adult mouse pituitary to provide a comprehensive view of cellular composition across all lobes.

We therefore applied Visium (10x Genomics) spatial transcriptomics to 12 adult mouse pituitary sections to measure gene expression across all lobes.

Although the Visium platform provided limited cellular resolution in the anterior lobe, it allowed more detailed characterization of the less densely packed posterior lobe, a region directly innervated by hypothalamic magnocellular neurons. Dimensionality reduction of the transcriptomic output revealed nine spatial clusters, with posterior lobe-enriched spots primarily localized within cluster 6. Marker gene expression analysis and cell-type deconvolution showed that cluster 6 comprised pituicytes in about 50% of cells, along with pericytes, endothelial cells, and macrophages, as expected. Importantly, we detected magnocellular neuron-specific axonal transcripts in high abundance, namely Avp and Oxt-encoded mRNAs. This observation supports the hypothesis that arginine vasopressin and oxytocin are not only transported, stored, and released as peptides from the pituitary, but also actively synthesized there, perhaps on demand. Further, we identified a similar gene expression profile between pituicytes and tanycytes, an ependymal subpopulation of cells localized along the hypothalamic third ventricle.

Overall, this dataset provides the first spatially resolved transcriptomic framework of the mouse posterior pituitary and a resource for future studies of pituitary organization and cell-cell signaling within it.

FINANCIAL SUPPORT: The study is funded by NCN, Opus 19 project no. 2020/37/B/NZ3/03633.

## S14.3. ROLE OF GLIAL PITUICYTES IN REGULATING NEUROHYPOPHYSEAL VASCULAR PERMEABILITY IN ZEBRAFISH LARVAE

Athul R. Ramesh, Savani Anbalagan

*Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poland*

The blood-brain barrier (BBB) serves as a critical safeguard for the central nervous system (CNS), but it also presents a formidable obstacle to effective therapeutic drug delivery. Neurohypophysis (NH), a circumventricular organ, circumvents the BBB due to its specialized fenestrated vasculature, which allows selective molecular permeability. The molecular mechanisms that regulate vascular permeability in the NH are relatively unknown.

Our research investigates the genetic and molecular pathways underlying NH vascular permeability in zebrafish, with the aim of identifying key regulators that could be leveraged to modulate BBB permeability.

Towards this goal, we have been employing pharmacological and genetic perturbations to explore the roles of candidate molecules that could regulate neurohypophyseal vascular permeability.

We found that pharmacological and genetic perturbation of Wnt signaling can regulate neurohypophysis vascular permeability. Through transcriptomic datamining, we have identified some Wnt regulators as a potential candidate genes and signaling pathways that govern NH-specific vascular fenestrations and permeability.

Our studies are expected to reveal a novel role of Wnt regulators in the vascular development of a key neuroendocrine interface conserved in vertebrates.

FINANCIAL SUPPORT: SONATA-BIS 2020/38/E/NZ3/00090.

## S14.4. NEUROBIOLOGICAL APPLICATION OF TEMPORAL COPULATORY PATTERNS OF FEMALE RAT SEXUAL BEHAVIOR

John C. Oyem, Jauma Lalanza, Patty Huijgens, James Edgar McCutheon, Roy Heijkoop, Eelke Snoeren
*UiT The Arctic University of Norway*

Female rat sexual behavior is a rewarding activity essential for reproduction and species continuity. However, previous studies have been limited by relying on a set of specific parameters, leaving significant gaps in our understanding of female rats' temporal copulatory patterns. Additionally, the neural mechanisms underlying reproductive behaviors remain fully unraveled.

Our study developed a new behavioral tool for analyzing female rats' copulatory patterns. We applied this tool to enhance the interpretation of intervention studies, particularly those targeting the ventral tegmental area (VTA), a brain region implicated in reward and motivation.

We examined two groups of female rats (fully receptive *vs.* less receptive), that hormonally primed with estradiol benzoate (EB) alone or EB + progesterone (P) across six paced mating tests. This design allowed us to compare the effects of hormonal priming on female rats' copulatory patterns. Based on this experiment, we organized female copulatory behavior into bouts and then combined fiber photometry with a behavioral reward paradigm to investigate VTA neural responses during female rat sexual behavior.

Our findings revealed that female rats exhibit structured patterns of sexual bouts and time-outs. Fully receptive females displayed more frequent sexual bouts and shorter time-outs. Furthermore, neural activity in the VTA increased during the onset of paracopulatory behavior and at the end of the copulatory stimulation received by the female, indicating its role in regulating sexual bouts rather than time-out.

This demonstrated that copulatory patterns are organized into sexual bouts and time-outs. While sexual bouts reflect copulatory speed, time-outs indicate motivation to continue mating. Our findings further highlight the crucial role of the VTA in regulating distinct aspects of female rat sexual behavior, emphasizing the importance of detailed behavioral assessments in behavioral neuroscience research.

FINANCIAL SUPPORT: Financial support was received from Helse Nord (HNF1443–19) and the AKM fund of UiT The Arctic University of Norway and MSCA-IF.

## S14.5. SECOND TIME IS THE CHARM: K – OPIOID RECEPTORS CONTROL EXPRESSION OF SOCIAL MEMORY

Aleksandra Rzeszut[1], Julia Netczuk[1], Klaudia Misiołek[1], Łukasz Szumiec[1], Krystyna Gołembiowska[2], Zofia Harda[1], Barbara Ziółkowska[1], Jan Rodriguez Parkitna[1]

[1] *Maj Institute of Pharmacology Polish Academy of Sciences, Department of Molecular Neuropharmacology, Cracow, Poland*
[2] *Maj Institute of Pharmacology of the Polish Academy of Sciences, Unit II, Department of Pharmacology and Brain Biostructure, Cracow, Poland*

The κ-opioid receptor (KOR), activated by endogenous dynorphin peptides, is widely expressed across the brain and regulates transmitter release within areas relevant to emotional processing, thereby shaping social cognition and behavior. Previous studies have linked the dynorphin/KOR system to social memory, a core component of social cognition in group-living species. It was observed that inhibition of KOR signaling enhances partner recognition and KOR activation weakens it. However, the specific KOR-expressing neuronal populations that underlie these effects remain unknown.

We hypothesized that KOR signaling constrains social recognition by suppressing oxytocinergic or serotonergic circuits, such that loss of KOR signaling within these neuronal populations would improve social recognition and be associated with altered baseline monoamine levels.

Social memory was assessed in both sexes using a two-trial social recognition paradigm in mice with global prodynorphin deletion (Pdyn$^{-/-}$) or selective KOR removal from oxytocinergic (Oprk1OxtCre) or serotonergic (Oprk1Tph2CreERT2) neurons. Neurochemical analyses were performed in male and female mice by quantifying total tissue concentrations of NA, 5-HT, DA, and their metabolites (5-HIAA, DOPAC, HVA) in the striatum and prefrontal cortex using high-performance liquid chromatography.

Consistent with previous reports, male Pdyn (-/-) mice exhibited enhanced social memory; notably, this phenotype was also observed in female Pdyn (-/-). Selective deletion of KORs from oxytocinergic neurons enhanced social memory in females but not males, whereas serotonergic KOR deletion had no impact. Additionally, female Oprk1OxtCre mice displayed increased baseline 5-HIAA/5-HT ratios in the prefrontal cortex and elevated baseline striatal NA levels.

Together, these findings demonstrate that dynorphin/KOR signaling constrains social recognition memory, and that in females, this effect is mediated through KORs on oxytocinergic neurons.

FINANCIAL SUPPORT: National Science Centre, Poland OPUS 2019/35/B/NZ7/03477.

## S15.1. BRAINTRAWLER: AN INTERACTIVE PLATFORM FOR EXPLORING MULTIMODAL DATA IN HUMANS AND MICE

Sophia Ulonska, Tobias Peherstorfer, Marvin Kleinlehner, Bader Al-Hamdan, Katja Bühler
*Biomedical Image Informatic Group, VRVis GmbH, Vienna, Austria*

The integration and analysis of multi-modal brain data promise to enhance our understanding of the brain. Enabling researchers to access and explore this data is essential for advancing neuroscience research.

With BrainTrawler (https://braintrawler.vrvis.at/), a free, web-based tool, we aim to provide low-threshold access to the integrated data, consisting of the BrainTACO resource (Brain Transcriptomic and Connectivity Data), several brain activity datasets and predefined circuits based on the NIMH Research Domain Criteria (RDoC). BrainTrawler allows for interactive exploration, visualization, and analysis of the integrated data. Furthermore, we offer a novel method for circuit mining.

Diverse public datasets for humans and mice were integrated and mapped to the Allen Human Reference Atlas (AHRA) and the Allen Mouse Brain Atlas (AMBA). Methods for interactive querying of gene expression, connectivity, and activity across arbitrary brain regions were implemented. A special spatial indexing technique enables real-time user interaction with the data. Additionally, we developed a unique method to derive and analyze circuits from brain activity signals in real-time.

The interactive queries facilitate exploration from single-cell gene expression to connectivity and brain activity across various brain initiatives and species in a 3D context. The new circuit mining approach allows for the identification of differentially expressed genes in entire circuits or specific components. Furthermore, BrainTrawler aids in identifying potential target genes relevant to meso-scale brain circuits.

BrainTrawler aims to support data-driven insights and hypothesis generation for brain research. It also serves as a teaching tool for understanding brain-gene-function relationships. In summary, BrainTrawler provides accessible exploration of multi-modal brain data for students and researchers in academia and the pharmaceutical industry.

FINANCIAL SUPPORT: The VRVis GmbH is funded by BMK, BMAW, Tyrol, Vorarlberg, and Vienna Business Agency in the scope of COMET – Competence Centers for Excellent Technologies (911654) which is managed by FFG.

## S15.2. LARVALBRAIN: A MULTIMODAL DROSOPHILA RESOURCE FOR GENETIC TOOL DISCOVERY

Tobias Peherstorfer[1], Sophia Ulonska[1], Bader Al-Hamdan[1], Marvin Kleinlehner[1], Andreas Thum[2], Tilman Triphan[2], Katja Bühler[1]
[1] *VRVis GmbH, Vienna, Austria*
[2] *University of Leipzig, Germany*

The fruitfly larva is a prominent model organism in neuroscience research. Data for the Drosophila larva is available at different developmental stages, posing significant challenges to experiments involving different data modalities. The connectome of the first larval instar is often a starting point for studying the impact of neurons on behavior. However, connecting findings from the connectome to other data can be challenging and often requires both computational and anatomical expertise. However, genetic tool data, which allows scientists to manipulate neurons, is mostly available at the third larval instar.

We want to enable researchers without a computer science/bioinformatics background to connect findings in the larval connectome data to data from later developmental stages at a low threshold.

We apply computer science methods to large collections of images and anatomical structures to allow real-time queries. In addition, we use visual analytics methods to allow users to interactively explore and combine results.

Our software allows for real-time queries against drosophila larva data from different instars In particular, we have developed a novel approach for discovering potential split-line parents by mining large Gal4 light microscopy image collections from the 3rd larval instar. Additionally, we show an approach to link high-resolution electron microscopy neuron tracing to Gal4 staining data.

We demonstrate the web-based data exploration tool Larvalbrain and showcase how light and electron microscopy imaging data from different larval instars can be explored and searched interactively to inform future behavioral experiments. Our software enables researchers to easily access complex and multimodal neuroscientific data without requiring programming skills.

FINANCIAL SUPPORT: This research was funded by the Austrian Science Fund (FWF) larvalbrain 2.0 (doi: 10.55776/I4836).

## S15.3. PHARMACOLOGICAL AND RESTING STATE FMRI REVEAL OSTEOCALCIN'S EFFECTS ON MOUSE BRAIN REGIONS WITH HIGH GPR37 AND GPR158 EXPRESSION

Natalia K. Freus[1], Isabel Wank[1], Maximilian Häfele[1], Liubov S. Kalinichenko[2], Christian P. Müller[2,3], Sandra Strobelt[1], Andreas Ludwig[1,5], Silke Kreitz[1,4], Andreas Hess[1,4,5]

[1] *Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany*

[2] *Department of Psychiatry and Psychotherapy, University Clinic, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany*

[3] *Institute of Psychopharmacology, Central Institute of Mental Health, University of Heidelberg, Germany*

[4] *Institute of Neuroradiology, University Clinic Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany*

[5] *FAU NeW – Research Center for New Bioactive Compounds, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany*

Osteocalcin (OCN) as endocrine hormone acts in the brain through receptors GPR158 and GPR37, influencing monoamine neurotransmitter synthesis. OCN KO mice show increased anxiety and depression-like behavior. Above findings and decreased OCN levels in depressed patients suggest an involvement of OCN in mood disorders.

We used gene expression data from the Allen Brain Atlas for OCN's receptors and multiparametric MRI to investigate the effects of intravenously administered OCN on the wild type mouse brain. We investigated further if OCN induces characteristic brain states.

We calculated mean gene expression density for Gpr158 and Gpr37 per brain region. Pharmacological MRI (phMRI) and resting state functional MRI (rs-fMRI) were conducted using a 4.7 T MRI with simultaneous i.v. injection of OCN or saline into the lateral tail vein of mice. Dynamic functional RS connectivity was calculated after *vs.* before injection of OCN using multi-seed region analysis and a sliding-window approach. PhMRI elicited changes in relative cerebral blood volume (rCBV). Brain states were calculated using an agglomerative hierarchical clustering analysis with distance measure correlative and centroid as cluster links.

Brainstem, association cortex, basal ganglia, and limbic output are among the top 10% of OCN-modulated brain regions in all 3 analytical entities (phMRI, rs-fMRI, gene expression). Two major dynamic functional connectivity states (dFCS) emerged; one dFCS is present in control animals only and represents a "basic" state whereas the second dFCS is favored by OCN but still present in control animals, representing a more "salient" state.

Our study revealed that OCN modulates rCBV and resting state in the mouse brain by targeting brain regions with high Gpr158 and Gpr37 expression. OCN induces characteristic brain states shifting from "basic" to "salient", involving brain regions associated with anxiety and depression-like behavior.

FINANCIAL SUPPORT: I have nothing to declare.

## S15.4. COMPUTATIONAL RECONSTRUCTION OF EVOLUTIONARY SELECTION SHAPING SOCIO-AFFECTIVE TRAITS IN HUMAN BRAIN NETWORKS

Lukasz Piszczek[1], Clara Fazzari[1,2], Sophia Ulonska[3], Raphael Schürger[1], Nora Popescu[1], Maja Adel[1], Kaja Moczulska[1], Katja Bühler[3], Wulf Haubensak[1,4]

[1] *Department of Neuronal Cell Biology, Center for Brain Research, Medical University of Vienna, Vienna, Austria*

[2] *Department of Biology, University of Rome Tor Vergata, Rome, Italy*

[3] *Biomedical Image Informatics, VRVis GmbH, Vienna, Austria*

[4] *Research Institute of Molecular Pathology (IMP), Vienna Biocenter (VBC), Vienna, Austria*

Public ancient genomes and open multimodal brain resources enable in silico approaches to understand human brain evolution. However, such progress is limited by a lack of integrated multimodal datasets and interpretable analysis pipelines that connect molecular change to brain systems and cognition.

Here, we aim to present a holistic workflow that maps multigenic evolutionary signals onto cognitive domains, functional brain networks, brain cell types, and candidate molecular mechanisms, with a focus on socio-affective traits.

We integrated expanded evolutionary genetic data with synthetic fMRI resource comprised of human task-derived functional networks, pre-clustered into concise psychological domains. We then employed a Genetic Algorithm for Generalized Biclustering (GABi) to mine the multigenic evolution across brain space. The discovered biclusters are then explored for cell-type expression signatures, followed by Gene Ontology enrichment to infer candidate mechanisms.

The workflow detected peaks of adaptive selection in socio-affective networks supporting social interaction (including language) and social concepts (including theory of mind), with signals distributed across hominid, early hominin, and anatomically modern human ancestry. Associated cell-type signatures span

diverse excitatory (glutamatergic) and inhibitory (GABAergic) neuronal populations and non-neuronal cell types. Enriched molecular functions implicate cell signaling, synaptic organization, and neuronal morphology, consistent with evolutionary tuning of circuit assembly and function.

This mesoscale "computational archaeology" framework bridges bottom-up genomic/cellular evidence and top-down systems neuroscience, enabling scalable reconstruction of putative archaic brain functional features. The same approach can be adapted to screen brain functional traits relevant to neuropsychiatric risk or to compare evolutionary trajectories across non-human phylogenies.

FINANCIAL SUPPORT: Clara Fazzari was supported by the Erasmus+ Students Traineeship a.a. 2023-2024. VRVis GmbH is funded by BMK, BMAW, Tyrol, Vorarlberg, and Vienna Business Agency in the scope of COMET – Competence Centers for Excellent Technologies (911654), which is managed by FFG. This work was supported by the FFG FEMtech Praktika to Lena Reitinger. WH received funding from the Research Institute of Molecular Pathology (IMP), Boehringer Ingelheim, and the Austrian Research Promotion Agency (FFG). This research was funded in part by the Austrian Science Fund (FWF COE-16B).

## S15.5. CONSENSUS SHARED ALIGNMENT (COSA): FAIR AND ROBUST ALIGNMENT OF SUBJECTS WITH HETEROGENEOUS BRAIN ACTIVITY PATTERNS THROUGH A MULTI-METHOD META-APPROACH

Julian Kędys, Cezary Mazurek

*Department of Digital Medicine, Poznań Supercomputing and Networking Center, Polish Academy of Sciences, Poznań, Poland*

Studying neurodevelopmental conditions of complex clinical phenotypes is among the most critical and simultaneously the most challenging endeavours in modern neuroscience. Population-level alignment and dimensionality reduction of brain-activity time series enable cross-subject comparisons – essential for productive, rigorous research in the domain – but a single alignment method can favour subjects whose dynamics match its inductive bias, diluting subgroup-specific temporal structure in the shared space.

We construct a controllable, transparent meta-alignment that fuses complementary population methods to obtain a shared latent representation that is robust and comparatively fair across heterogeneous cohorts.

We propose Consensus Shared Alignment (CoSA). For each method (e.g., group principal component analysis (PCA), independent component analysis (ICA), shared response model (SRM), multi-set canonical correlation analysis (MCCA)), we estimate low-dimensional loadings and align them to a common reference basis using an orthogonal Procrustes map. Components are matched one-to-one with a Hungarian algorithm and signs are harmonised. Aligned loadings are fused with non-negative weights that sum to one, chosen from cross-method agreement and stability diagnostics. Subject latent trajectories are then computed and standardised within subject. CoSA reports permutation-based reproducibility, temporal continuity and subsample-stability checks.

On whole-brain rodent imaging data (controls + autism-model subtypes), CoSA yielded stable shared coordinates without single-method dominance. Relative to individual methods, CoSA increased cross-subject reproducibility while preserving subtype-specific temporal structure, enabling direct subgroup-faithful comparisons.

Multi-method consensus alignment can reduce inductive-bias effects in population embeddings and provides practical diagnostics for reliability and control, supporting fairer analyses in heterogeneous neuroimaging data.

FINANCIAL SUPPORT: None.

## S16.1. EFFECTS OF CUE TRANSPARENCY ON LANGUAGE CONTROL IN PURE- AND MIXED-LANGUAGE CONTEXTS

Hanna Cwynar, Kalinka Timmer
*University of Warsaw, Faculty of Psychology, Warsaw, Poland*

Bilinguals typically incur costs when switching compared to repeating a language, a phenomenon attributed to bilingual language control (BLC). To date, most research used arbitrary cues to signal the response language, and studies examining the effects of different cues on BLC have focused on mixed-language contexts.

We investigated how different cues affect BLC across pure- and mixed-language contexts to understand the impact of cue transparency and processing on language switching tasks.

31 unbalanced Polish (L1)–English (L2) bilinguals named pictures in L1 and L2 as signalled by auditory arbitrary tone cues (low *vs.* high) *vs.* transparent question cues ("Co?" *vs.* "What?") in pure- and mixed-language blocks, while reaction times (RTs) and electrophysiology (EEG) were recorded.

In mixed-language blocks, behavioral switch costs showed 37ms reduction with question relative to tone cues, supported by cue-locked N2 modulations showing greater switch costs in tones than questions. In addition to greater cue-N2 amplitudes for tones than questions, cue-N1 amplitudes were also greater for tones, suggesting more selective attention (N1) and cue updating (N2) may be required for tones. We also observed cue-N2 language-specific temporal differences in questions (L1 earlier than L2) but not tones. Yet, in pure-language blocks, cue-N1 and cue-N2 patterns by cue type and language partially matched those in mixed blocks, while RTs were unaffected by cue type. This suggests that some neural effects in mixed blocks may reflect cue processing rather than BLC, with behavioral cue-transparency facilitation emerging only when cues are necessary for task performance.

Results suggest that BLC is shaped by cue processing and transparency. We propose cues that resemble input encountered in talk-in interactions, directly signalling the language and the action that achieves the goal of communicating in that language, may reduce the apparent need for control commonly found with arbitrary cues.

FINANCIAL SUPPORT: The research was supported by the National Science Centre (Narodowe Centrum Nauki) through the OPUS grant (2022/45/B/HS6/01931) awarded to Kalinka Timmer.

## S16.2. THE DIFFERENCES BETWEEN BILINGUALS' LANGUAGES IN CONVERSATIONAL TURN-TAKING

Wiktoria Ogonowska, Kalinka Timmer
*University of Warsaw, Faculty of Psychology, Warsaw, Poland*

The mechanisms underlying bilingual language processing are understudied in conversational contexts. Previous research focused on isolated speech production, but in real life, we produce speech in response to other people's utterances. Recent conversational studies with monolinguals have revealed that monolinguals prepare their responses during the interlocutor's turn, but they only begin speaking when the utterance is finished.

This study aims to examine conversational turn-taking processes in bilingual speakers and explore the similarities and differences between their languages.

Polish (L1) – English (L2) bilinguals completed a speech production task in both languages. They heard general knowledge questions and answered them aloud. The critical information needed to answer the question was presented either in the middle (EARLY) or at the end (LATE). Behavioral and EEG data were collected.

Bilinguals were more accurate and faster in L1 than in L2. Bilinguals' accuracy was similar between EARLY and LATE questions, but they answered EARLY questions faster. The Late Positivity (LP) ERP component was present in reaction to the critical word compared to the non-critical word. There was no difference between languages in LP when the critical word appeared EARLY; when the critical word appeared LATE, the amplitude of the LP was larger for L1 than for L2.

The turn-taking pattern previously observed in monolinguals was replicated in both the L1 and L2 of bilinguals. Although previous research has shown slower word retrieval in L2, results revealed that speech planning in L2 is not delayed (LP). This suggests that auditory language cues directly activate the heard language and facilitate speech planning in L2. The difference between languages at the end of the question suggests that the longer RTs for L2 are caused by the later stages of production connected to articulation, and not by the speech planning.

FINANCIAL SUPPORT: This work was supported by grants awarded to Kalinka Timmer from the National Science Centre (Narodowe Centrum Nauki) with an

OPUS (2022/45/B/HS6/01931) and the Faculty of Psychology, University of Warsaw, from the funds awarded by the Ministry of Science and Higher Education in the form of a subsidy for the maintenance and development of research potential in they year 2026 (501-D125-01-1250000 zlec. 5011000677).

## S16.3. LATE FRONTAL POSITIVITY (LFP) AS AN INDICATOR OF METAPHOR PROCESSING IN POLISH: A STUDY USING NONPARAMETRIC CLUSTER-BASED PERMUTATION ANALYSIS

Kamil Wałczyk

*College of Interdisciplinary Individual Studies, University of Silesia in Katowice, Katowice, Poland*

This work addresses the problem of how the human brain processes mental metaphors in verbal phraseologisms in the Polish language. It engages with the embodied cognition framework to understand dynamic changes in the brain's electrical activity during language comprehension.

The study aimed to investigate neural processing differences between four contexts of action verbs: motor literal (physical action, e.g., 'throws a ball'), phraseological (common metaphor, e.g., 'throws an idea'), unknown figurative (novel metaphor, e.g., 'throws a wish'), and mental literal (abstract control, e.g., 'considers a decision'). The central research question was whether the neural representation of these contexts would differ and if phraseological contexts are processed differently than literal ones.

N=34, right-handed participants read 30 sentences per condition while EEG signals were recorded. Stimuli was presented in 1800 ms time window. Artifact-free epochs (-200 to 1500 ms) were time-locked to stimulus onset. We utilized nonparametric cluster-based permutation analysis to identify significant spatiotemporal differences in event-related potentials (ERPs) without specifying a priori boundaries, effectively correcting for multiple comparisons.

An amplitude decrease in the sequence: phraseological > unknown figurative > motor literal > mental literal. A robust effect distinguished the phraseological condition from mental literal and motor literal conditions, corresponding to a spatiotemporal cluster maximal over frontal/central areas (550–700 ms). This represents a Late Frontal Positivity (LFP). A second effect (250–350 ms) differentiated phraseological and unknown figurative conditions from the mental literal condition.

Results suggest a two-stage mechanism for processing non-literal action verbs. The LFP indicates a specific cognitive cost for the phraseological condition. Findings support the embodied cognition framework and demonstrate the utility of cluster-based analysis.

FINANCIAL SUPPORT: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## S16.4. LANGUAGE-SELECTIVE INHIBITION OF NEGATIVE AFFECT IN BILINGUAL WORD PRODUCTION

Rafał Jończyk[1,2], Guillaume Thierry[3]

[1] *Faculty of English, Adam Mickiewicz University, Poznań, Poland*

[2] *Cognitive Neuroscience Center, Adam Mickiewicz University, Poznań, Poland*

[3] *School of Psychology and Sport Science, Bangor University, Bangor, Wales*

Semantic processing of negative content appears to be reduced when bilinguals read for comprehension in their second language (L2). However, this phenomenon has seldom been studied in production or when individuals listen to words, and the underlying mechanism remains unknown.

We investigated whether the dampening of negative emotional content applies in production and examined its underlying mechanism, specifically testing inhibitory control during L2-to-L1 translation of negative words by Polish-English bilinguals.

Polish–English bilinguals translated written (exp. 1, n=35) or spoken (exp. 2, n=35) negative and neutral words either from L2–to–L1 or L1–to–L2. We analyzed behavioral accuracy and response times, Event-Related Potentials (ERPs), and neural oscillation power via hierarchical linear modeling (LIMO; Pernet 2011, 2015).

Bilinguals were less accurate and slower when translating into L2 than into L1, but there was no interaction between target language and word valence in either experiment. Hierarchical linear modeling of of ERPs showed higher positivity for negative than neutral words only in the L2–to–L1 direction (production in Polish) and only in experiment 1, between 300–750 ms after stimulus onset. Hierarchical linear modeling of neural oscillation power revealed a two-step inhibitory process: stimulus integration (240–365 ms over parietal electrodes) and speech preparation (620–800 ms over anterior sites).

We provide initial evidence that emotion modulation by language in bilinguals involves inhibitory control. The absence of such effects in experiment 2 may relate to the evolutionary precedence of auditory over visual language processing and the production context used. Our results point to inhibitory control as a possible mechanism underlying the commonly observed attenuation of negative emotion in a second language.

FINANCIAL SUPPORT: National Science Center (2020/37/B/HS6/00610).

## S16.5. NEONATAL LEARNING OF SPEECH SOUND PATTERNS

András Ambrus[1,2], Silvia Polver[1,3,4], Brigitta Tóth[1], Gábor Háden[1]

[1] *HUN-REN Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Budapest, Hungary*

[2] *Department of Cognitive Science, Faculty of Natural Sciences, Budapest University of Technology and Economics, Budapest, Hungary*

[3] *GRAMFC, Inserm UMR1105, Université de Picardie Jules Verne, Amiens, France*

[4] *Department of Developmental Psychology and Socialisation, University of Padua, Italy*

The automatic capacity to extract, encode and utilize statistical properties of the ever changing sensory environment, known as statistical learning, is a fundamental ability already present from birth. Detecting longer recurring auditory patterns consisting of as many as 10 pure tones is proved to be present in newborn babies. Whereas pure tones only provide information about pitch transitions, speech includes a wealth of acoustic information.

This study investigated whether sleeping newborns detect regularities in speech-like sound sequences.

Using time-locked EEG measures, we compared 35 newborn babies' (up to 2 days of age) neural responses to regularly recurring and random pseudo-syllable sequences.

We found that neonates show distinct electrophysiological responses to regular versus random patterns, indicating that the neonatal auditory system is sensitive to structured syllable sequences.

These findings suggest that even at a very early age, infants can exploit redundancies in speech input, an ability underpinning later language acquisition. The present results provide an important step toward identifying early neural signatures of pattern learning in speech. Such responses may serve as potential biomarkers of language learning capacities in early infancy, offering a window into the developing mechanisms that support speech segmentation and grammar acquisition. Beyond their theoretical relevance, these results underscore the methodological value of the current paradigm: it provides a robust, non-invasive, and replicable tool for probing the infant brain's capacity to extract structure from continuous auditory input. This paradigm can be extended to assess individual differences and atypical trajectories, contributing to the early identification of infants at risk for language disorders.

FINANCIAL SUPPORT: BIO-PREPA (2024-1.2.2-ERA_NET-2025-00023) Biomarkers and Behavioral Probes for Preclinical Perinatal Asphyxia Funding: ERA-NET NEURON Cofund2, Horizon 2020 Grant Agreement No. 964215 Principal Investigator and Project Coordinator: Brigitta Tóth.

## S17.1. BEHAVIORAL PARADIGMS THAT ASSESS HIGHER-ORDER LEARNING ACROSS MULTIPLE SENSORY MODALITIES

Arnau Busquets-Garcia

*Hospital Del Mar Research Institute, Barcelona, Spain*

Understanding how the brain links events that were never directly paired is central to explaining complex learning, daily choices, belief formation, and the emergence of maladaptive perceptions. Higher-order conditioning, including sensory preconditioning, provides a powerful framework to investigate how neutral cues become meaningfully associated through incidental experience.

This talk will describe cutting-edge behavioral models, computational approaches, and neurobiological techniques to study higher-order conditioning processes using different sensory modalities.

We combined sensory preconditioning and second-order conditioning paradigms in mice with calcium imaging techniques and chemogenetics to evaluate the brain circuits involved in higher-order conditioning.

We successfully developed and odor taste sensory preconditioning task and demonstrated that neuronal projections from the Lateral Entorhinal Cortex to Basolateral Amygdala are crucial for this form of higher-order conditioning. We also set up light-tone sensory preconditioning and second-order conditioning paradigms and showing the involvement of hippocampal circuits. Finally, we are developing sensory precondi-

toning tasks using social-related cues in order to establish social higher-order conditioning paradigms allowing us to study the associated brain circuits.

Overall, our lab is advancing in the understanding of higher-order conditioning processes, which could help to better understand brain disorders accompanied by deficits in these complex cognitive processes.

FINANCIAL SUPPORT: ERC Starting Grant (ref. 948217) from the European Research Council.

## S17.2. INVESTIGATING THE ROLE OF THE HIPPOCAMPUS IN SENSORY PRECONDITIONING

Melie Talaron[1,2], Simon Killcross[2], Nathan Holmes[2], Etienne Coutureau[1]

*[1] Team Decision and Adaptation (DECAD), Univ. Bordeaux, CNRS, INCIA, UMR 5287, Bordeaux, France*

*[2] School of Psychology, UNSW Sydney, Australia*

Animals and people integrate information acquired at different times to respond appropriately in novel situations. Sensory preconditioning in rats models this integration and allows to study its neural substrates. In the medial temporal lobe, a network of brain regions is known to support sensory preconditioning. However, the precise role of the hippocampus remains unclear due to inconsistent protocols across studies.

To address this gap, the current project examines how the hippocampus contributes to sensory preconditioning by testing systematically its involvement in forming, integrating, and retrieving memories across the three stages of an auditory-visual protocol of sensory preconditioning.

Using pharmacological inactivation (infusions of GABA agonist muscimol or NMDA receptors antagonist DAP-V), we evidenced stage-specific contributions of the dorsal hippocampus, ventral hippocampus and the perirhinal cortex. Then, understand how the involvements of the dorsal and ventral hippocampus differ within the three stages of sensory preconditioning, we used calcium-based fiber photometry recording of the two sub-regions.

We demonstrated that the ventral hippocampus and Prh encode incidental sensory associations, while the dorsal, ventral hippocampus and Prh integrate and retrieve sensory and emotional memories. Preliminary results show that the dorsal and ventral hippocampus respond differently to the stimuli during memory integration.

These findings advance understanding of how the brain constructs flexible behavioral responses from temporally separated experiences, with implications for psychiatric disorders characterized by maladaptive memory integration.

FINANCIAL SUPPORT: This work was supported by the National Center of Scientific Research (France), the National Research Agency (France) “FLEXTIME” to EC and ANR-20-SFRI-0001 to MT, the Brain Research Foundation “Neurodon” to EC, and an Australian Research Council Future Fellowship (FT190100697) to NH. MT was awarded a “CDSN Programme Doctoral Normalien” PhD fellowship from “Ecole Normale Supérieure de Lyon” and from “Fondation de la Recherche Médicale” under the agreement FDT202504020727.

## S17.3. THE ROLE OF THE ENDOCANNABINOID SYSTEM IN INCIDENTAL ASSOCIATIONS: FOCUS ON INTERACTIONS WITH DOPAMINE SIGNALING

Unai B. Fundazuri[1,2], Marta Barrera-Conde[1], Elisa Rampini[1,2], Paula Gómez-Sotres[1], Macarena González-Portilla[3], Sandra Beriain[1], Arnau Busquets-Garcia[4], Guillaume Ferreira[2,5], Giovanni Marsicano[1,2]

*[1] INSERM, U1215 NeuroCentre Magendie, Bordeaux, France*

*[2] University of Bordeaux, Bordeaux, France*

*[3] Facultad de Psicología, Universitat de València, Valencia, Spain*

*[4] IMIM, Hospital del Mar Medical Research Institute, Barcelona, Spain*

*[5] INRAE, UMR 1286 Nutrition and Integrative Neurobiology, Bordeaux, France*

Reinforced conditioning enables individuals to predict future events, but many everyday behaviors rely on unreinforced associations between low-salience stimuli, known as Incidental Associations (IAs). IAs enhance predictive capacity in unstable environments and are conserved across species. To study them in rodents, sensory preconditioning tasks are used, where two low salience stimuli (S1/S2) are presented together in a preconditioning phase, followed by classical conditioning of S1 with a potent reinforcer. As a result, subjects present a direct response to the S1 stimulus, but also display mediated responses to the S2 stimulus never explicitly reinforced, indicating IA formation. Using a sensory preconditioning task with

light (S1) and sound (S2) as sensory cues, we showed that mice exhibit both direct and mediated responses, indicating IA formation. Previous work demonstrated that type-1 cannabinoid receptors (CB1R) are critical for this process. Global CB1R knock-out mice did not show mediated responses while direct response was unaltered. Dopamine signaling has also been implicated in IAs. Consistent with these findings, we found that mice lacking CB1R specifically in D1-receptor–expressing cells (D1R-CB1R-KO) fail to show mediated responses, identifying this CB1R population as essential for IA formation. Furthermore, pharmacological activation of CB1R using THC during short preconditioning facilitated IAs under conditions insufficient for controls. Similar effects were observed by increasing endogenous cannabinoid levels via inhibition of endocannabinoid degradation with JZL195. Notably, THC also induced mediated responses in D1R-CB1R-KO mice, but this effect was blocked by JZL195, indicating that D1R-CB1R cells are required for physiologically but not pharmacologically induced IAs.

FINANCIAL SUPPORT: Fondation pour la Recherche Médicale, FRM.

## S17.4. THE IMPACT OF SLEEP DEPRIVATION ON PSYCHOSIS-LIKE PERCEPTION IN MICE

Natalia Zernicka-Glover[1,2], Fernanda Teixeira Subtil[1], James MacRae[1], Katharina Schmack[1,3]

[1] *The Francis Crick Institute, London, United Kingdom*

[2] *University College London Medical School, London, United Kingdom*

[3] *Division of Psychiatry, University College London, London, United Kingdom*

Sleep deprivation can trigger psychosis-like states, including altered perception reflected in auditory hallucinations, however the underlying mechanisms are unclear. Dopaminergic signalling is disrupted after sleep deprivation and associated with psychosis.

We therefore aim to establish how sleep deprivation leads to psychosis-like perception, investigating striatal neuromodulatory signalling.

To test this, we established a sleep-deprivation paradigm where mice are prevented from sleeping through motion. To establish whether this paradigm induced measurable effects on behaviour, we measured prepulse inhibition (PPI) and open-field locomotion. To specifically assess psychosis-like perception, we used a previously established task where mice detect tones and express confidence by waiting for reward. Hallucination-like percepts (HALIPs) are defined as high-confidence false alarms. To relate sleep deprivation to neuromodulatory state, we conducted mass spectrometry metabolic analysis in striatum and cerebrospinal fluid.

Sleep-deprived mice showed altered PPI and initial hyperlocomotion, validating our sleep-deprivation paradigm. Remarkably, sleep-deprived mice reported more HALIPs, demonstrating increased psychosis-like perception. Preliminary metabolomic profiling revealed significant alterations in neuromodulatory and related pathways.

Our results indicate that sleep deprivation induces behavioural alterations and psychosis-like perception and indicate a role of neuromodulatory state. Ongoing fibre photometry experiments aim to uncover the mechanisms directly linking neuromodulatory state to altered perception caused by sleep deprivation.

## S17.5. NEW BEHAVIORAL APPROACHES TO STUDY POSITIVE PSYCHOTIC-LIKE STATES DRIVEN BY DELTA-9-TETRAHYDROCANNABINOL IN MICE

Irene Manzanares-Sierra, Júlia S. Pinho, Carla Ramón-Duaso, Silvia Castany, Arnau Busquets-Garcia

*Hospital del Mar Research Institute of Barcelona, Barcelona, Spain*

Cannabis is the third most consumed drug worldwide, and its use has been associated with an increased risk of psychopathology. However, the neurobiological mechanisms underlying positive psychotic-like states remain poorly understood, limiting pharmacological options. Recently, higher-order conditioning behavioral paradigms have been proposed as a translational model of positive psychotic-like behaviors. In rodents, these processes can be investigated using sensory preconditioning paradigms, in which associations between neutral stimuli later evoke conditioned responses through mediated learning (ML). Increasing these associations promotes a transition from ML to reality testing (RT), a process commonly impaired in psychotic-like states.

This study aimed to establish a visual-auditory sensory preconditioning task in mice to model cannabis-induced positive psychotic-like alterations and to investigate the impact of cannabis in social perception.

We established a novel light–tone sensory preconditioning task in male mice, along with a RT protocol involving extended preconditioning associations. Mice received an acute administration of Δ9-tetrahydrocannabinol (THC; 1 mg/kg), alone or with the cannabinoid receptor type-1 (CB1R) inverse agonist rimonabant (2 mg/kg). Social perception was assessed by evaluating discrimination between a THC-treated conspecific and a neutral one.

Acute THC administration disrupted RT, indicating altered processing of internal sensory representations. This impairment was reversed by Rimonabant, demonstrating the CB1R role in this effect. Preliminary findings suggest that male adolescent mice can discriminate between a THC-treated conspecific and a neutral one, an ability impaired under THC treatment.

Using these behavioral paradigms, future experiments will investigate the molecular mechanisms underlying THC-induced psychotic-like effects, focusing on mitochondrial-associated pathways, to identify novel therapeutic targets for psychiatric disorders.

FINANCIAL SUPPORT: Caixa Health Research Call 2024: HR23-00793.

## S18.1. PHARMACOLOGICAL FMRI AND STANDARISED CLOUD-BASED WORKFLOWS FOR TRANSLATIONAL NEUROIMAGINING

Diana Cash, Michel Mesquita

*BRAIN Centre, King's College London and L&M DataScience GmbH, United Kingdom*

This joint talk will present an integrated view of pharmacological functional MRI (ph- fMRI) and cloud-based analysis platforms as complementary tools for translational neuroscience and CNS drug development. Drawing on experience from the BRAIN Centre's preclinical imaging programmes, the first part will outline how ph-fMRI can be used to map systems-level drug effects across circuits implicated in neurodegeneration, pain, neuroinflammation, trauma and stroke. The emphasis will be on study-design principles (choice of paradigms, anaesthesia versus awake imaging, dosing strategies and longitudinal readouts), as well as on the derivation of robust imaging biomarkers that support mechanism-of-action studies and back-translation between animal models and human cohorts. The second part will focus on the standardisation and scaling-up of such imaging approaches using cloud-native infrastructures. Using the neuroPhINDr platform as an example, the talk will describe how MRI and corroborative modalities (such as EEG and optical readouts) can be embedded in harmonised, AWS-hosted workflows that combine automated preprocessing, multimodal registration and integrated quality- control metrics. Project-level dashboards for tracking recruitment, data acceptance and pipeline performance will be highlighted as practical tools for managing complex, multi- centre studies. Throughout the presentation, particular attention will be paid to reproducibility and FAIR data principles, including the use of containerised analysis environments, standardised data structures and transparent reporting. By linking sophisticated ph-fMRI methodology with rigorously engineered, cloud-based pipelines, the talk will illustrate a realistic route towards more consistent, sharable and clinically relevant neuroimaging in both academic and industry settings.

## S18.2. OF MICE AND MECHANISMS: CONVERGENT AGGRESSION, DIVERGENT BIOLOGY

Marija Petrinovic

*King's College London, London, England*

Aggression is a common feature of neurodevelopmental conditions, but whether it reflects shared or distinct underlying mechanisms remains unclear. We show that similar increases in aggression can arise from divergent circuit-level activity. Importantly, these differences are accompanied by selective sensitivity to pharmacological interventions. Together, these findings demonstrate that convergent behavioral phenotypes can mask fundamentally distinct biological substrates, with important implications for targeted therapeutic strategies.

## S18.3. HIGH-THROUGHPUT OPTICAL MICROSCOPY APPROACHES FOR FUNCTIONAL IMAGING AND 3D RECONSTRUCTION OF NEURAL TISSUE

Julia Niemczycka, Mateusz W. Kucharczyk

[1] *Cancer Neurophysiology Group, Polish Centre for Technology Development, Łukasiewicz-PORT, Wrocław, Poland*

[2] *Biophotonics and Electrophysiology Lab, Imaging Laboratories, Center for the Development of Therapies for Civilization and Age-Related Diseases, Jagiellonian University Medical College, Kraków, Poland*

[3] *Wolfson Sensory, Pain and Regeneration Centre, King's College London, England*

Dr. Kucharczyk will introduce a practical overview of high-throughput linear and non-linear fluorescence microscopy approaches enabling (A) rapid functional sampling and (B) large-scale anatomical reconstruction across the peripheral and central nervous systems. A) Functional imaging pipelines will be demonstrated across custom-built and commercial wide-field, confocal, confocal endoscopy, and two-photon microscopes, illustrating how genetically encoded calcium indicators permit in vivo monitoring of neuronal activity in both the PNS and CNS. B) High-resolution volumetric imaging strategies will be showcased using confocal, two-photon, and light-sheet modalities, including the first Polish benchtop mesoSPIM platform developed in our laboratory.

Subsequently, PhD student Julia Niemczycka will present original datasets, including whole-organ 3D reconstructions of peripheral innervation paired with molecular profiling. A case study will revisit the specificity of transgenic reporter lines, revealing the anatomy and physiology of deep-tissue peptidergic afferents. Examples will include in vivo sensory-neuron calcium imaging in health and disease using wide-field and two-photon microscopy, as well as mesoscale reconstructions and practical experience gained during the construction of the mesoSPIM light-sheet microscope.

## S18.4. SYSTEMIC ZOLPIDEM ADMINISTRATION REDUCES KCL-INDUCED CSD FREQUENCY IN NAÏVE SPRAGUE-DAWLEY RATS

Karolina Nowalińska[1,2], Monika Marcinkowska[2], Lucyna Pomierny-Chamioło[3], Bartosz Pomierny[1], Mateusz Kucharczyk[1]

[1] *Department of Toxicology, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

[2] *Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

[3] *Department of Toxicological Biochemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

Cortical spreading depolarisation (CSD) contributes to secondary injury following ischemic stroke primarily by promoting expansion of the penumbra and recruiting peri-infarct tissue into the ischemic core, inducing metabolic exhaustion, and disrupting perfusion, worsens prognosis. Since GABAergic inhibition potently regulates neuronal excitability, we investigated whether zolpidem – a selective α1-subunit GABA A receptor positive allosteric modulator – affects KCl-induced cortical neuronal dynamics in vivo. Simultaneous electrophysiological (DC shift) and laser Doppler hemodynamic (cerebral blood flow, CBF) recordings from the rat cortical surface before and after intraperitoneal zolpidem administration. We also measured neurovascular coupling by looking at the DC wave and Doppler CBF changes. Zolpidem significantly reduced the frequency of KCl-induced CSD events compared to saline controls, with each DC shift corresponding to a matching CBF wave. Zolpidem did not markedly impact either DC or CBF rise and decay times, which indicates that it may not directly influence the dynamics of individual CSD wave. Despite unchanged wave shape, zolpidem modulated neurovascular coupling – the DC-CBF peak delay (normalized to baseline) was significantly reduced in drug-treated animals compared with saline-injected, indicating altered vascular responsiveness. Moreover, zolpidem induced a reduction in heart rate 10 minutes post-injection. Additionally, in middle cerebral artery occlusion (MCAO) rat model, systemic zolpidem effectively normalized extracellular glutamate levels measured through in vivo microdialysis, which may be associated with smaller amount of CSD waves. Together, these findings support a role for selective α1-GABA A receptor potentiation in attenuating pathological CSD activity and altering associated neurovascular responses. This identifies zolpidem as a candidate therapeutic modulator of CSD-mediated secondary injury processes relevant to ischemic stroke.

FINANCIAL SUPPORT: This research was funded by National Science Centre, Poland grant: 2018/30/E/NZ7/00247 and 2022/47/D/NZ4/02676 (electrophysiological recordings). The publication was created with the use of equipment co-financed by the qLIFE Priority Research Area under the program "Excellence Initiative—Research University" (no. 06/IDUB/2019/94) at Jagiellonian University. The studies were performed with the use of research infrastructure financed by the European Union with the Smart Growth Operation Programme, POIR 4.2 project no. POIR.04.02.00–00-D023/20.

## S18.4. NEUROPROTECTIVE POTENTIAL OF ZOLPIDEM IN ISCHEMIC STROKE: MOLECULAR MECHANISMS AND ELECTROPHYSIOLOGICAL RECORDINGS

Karolina Nowalińska[1], Monika Marcinkowska[2], Lucyna Pomierny-Chamioło[3], Bartosz Pomierny[1,6], Mateusz Kucharczyk[1,4,5]

[1] *Department of Toxicology, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

[2] *Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

[3] *Department of Toxicological Biochemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

[4] *Wolfson Sensory, Pain and Regeneration Centre, King's College London, UK*

[5] *Cancer Neurophysiology Group, Łukasiewicz Research Network – PORT, Polish Centre for Technology Development, Poland*

[6] *BioImaging Laboratory, Center for the Development of Therapies for Civilization and Age-Related Diseases (CDT-CARD), Jagiellonian University Medical College, Kraków, Poland*

This talk will outline the neuroprotective potential of zolpidem in ischemic stroke, integrating pharmacological findings with novel functional and anatomical imaging approaches for nervous system studies. Using a preclinical MCAO model, we demonstrated that early, low-dose zolpidem administration reduces infarct volume and improves neurological and motor outcomes. These effects are interpreted alongside analyses of GABAergic signaling, glutamate dynamics, and chloride homeostasis. Furthermore, we investigated cortical spreading depolarizations using electrophysiological recordings. Currently, we are developing a protocol to visualize CSD using functional magnetic resonance imaging, along with a setup for continuous recordings of optogenetically induced CSD. Together, these approaches highlight how combining pharmacology with cutting-edge imaging can advance our understanding of stroke pathology and recovery mechanisms.

## S18.5. INTEGRATIVE MRI ANALYSIS PIPELINES FOR MULTIMODAL AND SPATIAL OMICS DATA

Eugene Kim

*Institute of Psychiatry, Psychology & Neuroscience, Brain Centre, King's College London, London, UK*

This talk will present an end-to-end MRI image analysis framework designed for integration with high-dimensional imaging and omics datasets. It will outline key stages of the pipeline, including preprocessing and artefact correction, intra- and inter-subject registration across multiple MRI modalities, and systematic mapping of quantitative MRI readouts onto external data spaces such as spatial transcriptomics. Particular emphasis will be placed on robust co-registration strategies, harmonised anatomical references and the use of reproducible, containerised workflows that facilitate cross-centre applications. The presentation will also cover quality control procedures and data- structure standards, illustrating how carefully designed pipelines enable meaningful fusion of MRI with optical and molecular information in both basic and translational neuroscience.

## S18.5. THE BRAIN BEYOND THE ANEURYSM: FROM MORPHOMETRY AND NETWORKS TO TREATMENT EFFECTS AND TRANSLATIONAL BRIDGES

Kornelia M. Kliś

*Department of Neurosurgery and Neurotraumatology, Jagiellonian University Medical College, Kraków, Poland*

Intracranial aneurysms are traditionally evaluated and managed primarily on the basis of their estimated risk of rupture. However, growing evidence indicates that the presence of an aneurysm may be associated with broader neurological consequences, including subtle cognitive deficits and an increased risk of psychiatric disorders.

This talk addresses the hypothesis that intracranial aneurysms are linked to measurable alterations in cortical structure and large-scale brain network organization, potentially mediated by neuroinflammatory processes.

A multimodal neuroimaging approach is applied, combining cortical morphometry with advanced structural network analysis using Morphometric INverse Divergence (MIND) derived from magnetic resonance imaging data.

Comparisons are performed between patients with unruptured intracranial aneurysms and matched controls, focusing on differences in cortical thickness, surface area, and network topology. Additional analyses examine how these alterations vary depending on aneurysm size, anatomical location, and hemodynamic characteristics. A longitudinal component evaluates

patients undergoing aneurysm treatment to determine whether intervention mitigates or exacerbates the identified structural and network-level abnormalities. These findings are complemented by pilot neuropsychological assessments, offering preliminary insight into the clinical relevance of observed brain changes. Furthermore, translational strategies are outlined to bridge clinical findings with preclinical models, enabling controlled investigation of aneurysm progression and its impact on brain structure. Particular emphasis is placed on mechanisms related to neuroinflammation.

Overall, this work challenges the rupture-focused paradigm of aneurysm management and highlights the importance of considering broader effects on brain structure and function in both clinical practice and future research.

## S18.6. OPTIMIZING CNNS FOR DETECTING LOW AMPLITUDE EYE MOVEMENTS FROM FMRI

Zofia Sikorska[1,2], Jan K. Argasiński[1], Cemal Koba[1]

*[1] SANO Centre for Computational Personalised Medicine, AGH University of Kraków, Kraków, Poland*

*[2] Jagiellonian University, Kraków, Poland*

The fMRI signal from the eyes provides a proxy for gaze position, enabling retrospective analysis of gaze behavior and brain activity in open datasets. While prior work has successfully decoded gaze during large-scale tasks such as smooth pursuit and free viewing, detecting small-scale eye movements that are common in open-eyes resting-state (RS) paradigms remains challenging. The subtle movements during RS carry clinically relevant information but have much lower amplitudes than large-scale gaze tasks.

In this project, we aim to develop a model to detect low-amplitude eye movements from fMRI data.

We used simultaneous fMRI and eye-tracking data from 29 participants during a resting-state fixation scan. Mean gaze amplitude was ±1°, lower than large-scale tasks (±10°). We evaluated four strategies using a CNN model pretrained on large-scale gaze tasks: (1) directly applying the pretrained model to the RS dataset; (2) augmenting the original training set with RS data; (3) training a new model using only the RS dataset; and (4) transfer learning by freezing the pretrained backbone and fine-tuning on RS data using a composite loss (Smooth L1/Huber, $\delta$=1.0; Pearson weight=0.01; learning rate=1e-4; 40 epochs) to balance robust error minimization and trend capture.

Models pretrained on large-scale tasks systematically overestimated gaze amplitudes of RS data due to the amplitude mismatch. Training from scratch on the small RS dataset failed because of limited sample size and imbalance. The proposed transfer learning approach significantly improved performance, achieving a median Pearson correlation of 0.36±0.25 between predicted and true RS gaze data.

These results demonstrate that decoding small-scale spontaneous eye movements from resting-state fMRI is feasible but requires careful domain adaptation. Correlation-aware transfer learning effectively bridges amplitude differences, unlocking the potential to extract oculomotor information from existing resting-state fMRI datasets

FINANCIAL SUPPORT: This project was funded by National Science Center, Poland, grant no 2024/55/D/NZ5/02998. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 857533 and from the International Research Agendas Programme of the Foundation for Polish Science No MAB PLUS/2019/13. The project was created within the project of the Minister of Science and Higher Education "Support for the activity of Centers of Excellence established in Poland under Horizon 2020" on the basis of the contract number MEiN/2023/DIR/3796. We acknowledge Polish high-performance computing infrastructure PLGrid (HPC Center: ACK Cyfronet AGH) for providing computer facilities and supportwithin computational grant no. PLG/2025/01828.

## S19.1. PEPTIDERGIC CONTROL OF OSCILLATORY SOCIAL FORAGING PATTERNS IN NERVELESS PLACOZOANS

Mateusz Kostecki, Jules Duruz, Gaspar Jekely

*Centre for Organismal Studies, Heidelberg University, Heidelberg, Germany*

According to the chemical brain hypothesis, the evolution of the nervous system was preceded by systems based on chemical signaling, such as peptides. Placozoans are a group of nerveless, extremely simple marine animals that display characteristic, oscillatory, and partially social feeding behavior. During feeding,

the animals rhythmically expand and shrink their bodies to digest prey and absorb nutrients, a process that is controlled by peptidergic signaling. They represent an ideal model for studying the early emergence of behavioral coordination, the mechanisms of neuropeptidergic action, and the evolution of social behaviors.

The goal of our project was to explore the molecular basis of foraging behavior, guided by the assumption that peptide-evoked oscillatory behavioral patterns in Placozoa are driven by oscillations in cytoplasmic calcium concentration. We also investigated inter-animal coordination of oscillatory behavior and established an artificial evolution paradigm aimed at tracking changes in oscillatory patterns as the animals adapt to different temperature conditions.

To that end, we applied automated behavioral assays, machine-learning–based behavioral analysis, pharmacological approaches, and electron microscopy imaging to investigate the relationship between cytoplasmic calcium levels and oscillatory properties, as well as to observe the molecular structures connecting animals during social foraging.

We show that interventions targeting ion channels and pumps that regulate calcium transport into and out of the cytoplasm induce changes in oscillatory patterns consistent with our hypotheses. We also demonstrate that animals foraging socially coordinate their behavior and provide evidence that membrane-based connections of unknown nature may contribute to this effect. Furthermore, we present the results of an artificial evolutionary process in which animals adapt to lower temperatures by altering their oscillatory patterns.

We demonstrate that Placozoans are a very useful model for studying the coordination of behaviour by chemical signals, including neuropeptides, the evolution of social behaviour and behavioural adaptation to changing environmental conditions.

## S19.2. CYANOBACTERIAL NEUROTOXIN DABA ELICITS IMMENSE EXCITATION OF RETZIUS NEURON MEMBRANE THROUGH A NOVEL EXCITATORY PHENOMENON – TWO-STAGE DEPOLARIZATION

Svetolik Spasic[1,2], Marija Stanojevic[1,2], Marko Biorac[1,2], Nikola Jovanovic[2], Jelena Nesovic-Ostojic[1,2], Srdjan Lopicic[1,2]

[1] *Institute for Pathological Physiology "Ljubodrag Buba Mihailović", University of Belgrade, Belgrade, Serbia*

[2] *Faculty of Medicine, University of Belgrade, Belgrade, Serbia*

Neurodegenerative diseases are chronic, progressive and incurable neurological illnesses. Emerging evidence suggests that their origins lie in a complex interplay between genetic and environmental factors. The latter are of growing importance as life expectancy and exposure of people increase. A putative causative agent is 2,4 diaminobutyric acid (DABA), a product of ubiquitous cyanobacteria, whose neurotoxic potential is insufficiently examined.

We therefore examine the electrophysiological effects of DABA on Retzius neurons.

Effects of increasing concentrations of DABA (1, 3, 5 and 10 mM) on electrical properties of leech neuron membrane were investigated using intracellular sharp electrode recordings.

Our results show that DABA elicits a strong excitatory effect on neurons, causing extensive membrane depolarization several times larger than that induced by glutamate and aspartate or environmental excitatory amino acids. At concentrations of 3mM and higher, DABA induced a two-stage membrane depolarization, which is a novel excitatory phenomenon reported on our model. Depolarizing action of DABA is dependent on sodium ions and coupled with an increase of Retzius neuron membrane permeability. Blocking non-NMDA glutamate receptor with CNQX reduced the amplitude of the first stage, while inhibition of the transport system for neutral amino acids with L-Alanine markedly decreased the second stage of depolarization.

DABA produces substantial membrane depolarization, several times greater than that of other amino acids known for their excitatory effects, making it a potentially significant environmental factor in neurodegenerative diseases. This ubiquitous excitatory amino acid activates ionotropic glutamate receptors during the first stage and sodium-dependent transporters for neutral amino acids during the second stage of depolarization. These mechanisms initiate processes crucial for neurodegeneration – excitotoxicity, osmotic stress and energy depletion of neurons.

FINANCIAL SUPPORT: Ministry of Education, Science and Technological Development, project number 451-03-66/2025-03/200110.

## S19.3. SEX- AND AGE-DEPENDENT EFFECTS OF MATERNAL WESTERN DIET ON OFFSPRING NEURODEVELOPMENT

Aniqa Saiyara[1], Magdalena Chrószcz[2], Joanna Jastrzębska[1], Irena Smaga[1], Renata Pieniążek[1], Michał Korostyński[2], Małgorzata Frankowska[1], Małgorzata Filip[1]

*[1] Department of Drug Addiction Pharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

*[2] Department of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

Maternal diet during gestation and lactation is increasingly recognized as a critical determinant of offspring neurodevelopmental outcomes. Importantly, Western diet (WD) exposure at this crucial period predisposes offspring to cognitive deficits and long-term developmental disorders.

The impact of maternal WD throughout pregnancy and lactation on offspring behavior and region and age-specific brain molecular dysregulation at certain postnatal days (PND).

Female Wistar rats were fed either a control diet (CD) or a high-fat (WD) throughout gestation and lactation (14 weeks total). After weaning, both male and female offspring were maintained on a CD and assessed at (PND) 30, 60, and 90. Behavioral screening included the open field, elevated zero maze, self-grooming, marble burying, and social interaction tests. Furthermore, molecular mechanisms were examined using RNA sequencing of the hippocampus (HIP) and prefrontal cortex (PFC), followed by protein-level validation of selected targets using ELISA.

Maternal WD exposure increased body weight and obesity in male offspring and induced significant behavioral alterations at early developmental stages. At PND30, male progeny subjected to WD demonstrated notable changes in locomotor activity, anxiety-related behaviors, grooming, and social interaction, distinguished by varying sex-specific patterns. Moreover, molecular analyses revealed that male WD-offspring displayed alterations in HIP, with EDN1 dysregulation at PND30, alongside changes in KLF2, AGTRAP, VEGF, and eNOS at PND60. Conversely, female offspring exposed to WD exhibited VEGF dysregulation at PND60 in the PFC and eNOS changes at both PND30 and PND60.

To sum up, the above findings indicate that maternal (WD) exposure induces early, sex-dependent behavioral dysregulation accompanied by molecular alterations in specific brain regions critical for cognition and social behavior, supporting the concept of it as a contributor to neurodevelopmental vulnerability.

FINANCIAL SUPPORT: by research grant UMO-2022/45/B/NZ4/03951 from the National Science Centre, Kraków, Poland (to M. Filip), and partially funded by the Maj Institute of Pharmacology, Polish Academy of Sciences.

## S19.4. DETECTING NOVELTY IN DOMESTIC HORSES: P300-LIKE RESPONSES IN A PASSIVE AUDITORY ODDBALL PILOT STUDY

Ida Ilmer[1,2], Paweł Basoń[1,2], Michał Kuniecki[3], Magdalena Senderecka[2]

*[1] Doctoral School in the Social Sciences, Jagiellonian University, Kraków, Poland*

*[2] Centre for Cognitive Science, Jagiellonian University, Kraków, Poland*

*[3] Institute of Psychology, Jagiellonian University, Kraków, Poland*

Horses are large, non-laboratory mammals with strong flight responses and an underexplored neural basis of attention. Auditory attention is particularly relevant in this species, as unexpected sounds may trigger panic and dangerous reactions, posing risks to both horses and humans. This makes horses a valuable model for studying ecologically grounded attentional mechanisms and expanding knowledge of cognitive processing in phylogenetically distant, non-laboratory species.

This pilot study in comparative neurophysiology aimed to investigate neural correlates of auditory attention in horses using a passive auditory oddball paradigm. The main objective was to identify ERP components analogous to the human P3a/P300 novelty response occurring approximately 300 ms after unexpected stimuli.

This pilot study in comparative neurophysiology aimed to investigate neural correlates of auditory attention in horses using a passive auditory oddball paradigm. A total of 25 horses participated in the study. EEG was recorded using a two-electrode Bitalino amplifier during the presentation of 500 auditory stimuli, with 20% deviant tones. Stimuli were balanced across the horses: for half of the group, standard stimuli were 1000 Hz and oddball stimuli 1500 Hz, with the assignments reversed for the remaining horses. All stimuli were 50-ms lasting pure tones.

A paired-samples t-test showed a statistically significant difference between standard and deviant condi-

tions in the 300–400 ms time window after stimulus onset, t(24)=3.0874, p=.005, Mdiff=0.716 μV, 95% CI [0.237, 1.194 μV]. The results suggest that horses may exhibit P300-like neural responses to unexpected auditory stimuli.

Identification of a P300-like component in horses supports the applicability of neuroscientific methods to non-laboratory species in ecological conditions and provides a foundation for further research on attention and perception in large mammals, with potential implications for welfare and human–horse safety.

## S19.5. USING THETA WAVES TO MEASURE SELF-CONTROL IN THE DOMESTIC DOG

Ivaylo Borislavov Iotchev[1], Anna Kis[1], Márta Gácsi[2]

[1] *Institute of Cognitive Neuroscience and Psychology, HUN-REN Research Center for Natural Sciences, Budapest, Hungary*

[2] *Department of Ethology, Eötvös Loránd University (ELTE), Budapest, Hungary*

Dogs are considered a cognitively advanced species and anatomically posses a prefrontal cortex, but when and how the animals make use of higher cognitive control functions is not well researched.

We aimed to test if frontal-midline theta power, an established EEG concomitant of mental effort increases in dogs who are exposed to a self-control challenge.

We recorded half a minute long snippets of EEG from dogs who were either awaiting permission to eat a treat placed within their reach or were not engaged in any particular task. Both types of recording were matched in length and randomly sampled from within the same session, such that test and baseline recordings were obtained from similar condition: same room, same time of day, same number of people present.

Theta power measured at the front of the scalp midline was selectively elevated during the delayed gratification challenge.

Dogs who comply with orders of their owners appear to engage their prefrontal lobes, as opposed to relying on more ancient or automatic control processes.

FINANCIAL SUPPORT: LENDÜLET_2025-85.

## S20.1. WHEN ATTENTION DRIFTS, MEMORIES FAIL TO FORM BUT NOT TO DEGRADE

Philippe Blondé[1], Marcin Leszczyński[1,2]

[1] *Centre for Cognitive Science, Jagiellonian University in Kraków, Poland.*

[2] *Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York City, USA*

Mind wandering (MW) shifts attention from external input to internal thoughts, leading to a perceptual decoupling that disrupts episodic memory encoding. Although MW impairs memory performance, it remains unclear whether this impairment reflects a failure to encode information or a degradation of the fidelity of stored representations.

We tested whether MW affects the probability of successful encoding and the precision of encoded representations (color and location).

Thirty-four participants encoded 100 picture-item associations, and later identified the correct item in a forced-choice recognition task. Participants reproduced the item's original color and spatial location using circular responses scales. MW during encoding was measured with 14 thought probes (10-points Likert scale), while eye movements were recorded continuously. Response errors were modeled using a standard mixture model (von Mises + uniform), yielding estimates of retrieval probability (Pm) and mnemonic precision (κ).

MW significantly reduced episodic recall probability and increased response error for both color and spatial location, with a larger effect for location. Mixture modeling revealed that MW selectively decreased the probability of successful retrieval (Pm) and increased random responding, while leaving mnemonic precision (κ) largely unchanged. Oculomotor analysis showed that MW was accompanied by a decrease in saccade and fixation counts, together with an increase in blink frequency and saccade durations.

These findings suggest that MW creates an encoding failure rather than a degradation of stored representations. Consistent with the probability–precision dissociation framework, MW reduced the likelihood that an episodic trace was formed, but when encoding succeeded, representations retained normal fidelity. Thus, MW induces an "all-or-none" disruption of episodic encoding, suggesting an impaired engagement of encoding processes rather than a reduced representation.

FINANCIAL SUPPORT: Sonata 19: 2023/51/D/HS6/02920.

## S20.2. HOW SACCADIC EYE MOVEMENTS ORGANIZE AUDITORY PERFORMANCE IN NATURALISTIC CONTEXT

Katarzyna Jurewicz[1], Paweł Basoń[1], Krzysztof Basiński[2], Marcin Leszczyński[1,3]

[1] *Centre for Cognitive Science, Faculty of Philosophy, Jagiellonian University, Kraków, Poland*

[2] *Auditory Neuroscience Laboratory, Department of Psychology, Medical University of Gdańsk, Gdańsk, Poland*

[3] *Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York, USA*

Saccadic eye movements are one of the most common movements in primates. While their influence on neural activity has been traditionally studied in the visual system, saccades modulate ubiquitous networks that extend beyond the visual system. Despite these known dynamics, it is not clear how gazing behavior interacts with auditory cognition. Here, we studied auditory perception at different times relative to saccade onsets in freely viewing subjects.

Our study aims to define the impact of saccadic exploration on auditory perceptual threshold and performance across auditory tasks.

We conducted psychophysical experiments in which participants performed auditory tasks while freely viewing natural images. Each image was shown for 8 s and followed by content questions to encourage exploration. During a single image, 1–2 auditory probes were presented at random times through headphones. In Experiment 1, 10-ms pink noise was presented binaurally, with interaural time and level differences adjusted so it was perceived from the left or right. Participants reported the side via button press. In Experiment 2, a 6-ms tone (2 or 2.5 kHz) was presented monaurally to either ear, and participants indicated whether it was low or high.

Saccades away from sound elicited perceptual cost while saccades towards the sound elicited perceptual benefit in the task requiring integration of information from left and right ear (Exp. 1). The single-ear discrimination task also demonstrated the cost of saccading away from the sound (Exp. 2). However, no benefit could be observed here for the saccades towards the sound.

Our results suggest that saccades may support integration of information across ears or otherwise influence specific auditory processes during that task rather than uniformly influence auditory perceptual threshold. Alternative explanations will be tested with additional tasks aimed at dissociating perceptual and response-related processes.

FINANCIAL SUPPORT: National Science Centre, Poland, OPUS 2022/45/B/HS6/04097.

## S20.3. REWARD-BASED MODULATIONS OF SACCADIC EYE MOVEMENT KINEMATICS SHAPE THE TIME COURSE OF PRESACCADIC ATTENTION

Łukasz Grzeczkowski[1,2], Martin Rolfs[2]

[1] *Institute of Psychology, University of Social Sciences and Humanities, Katowice, Poland*

[2] *Department of Psychology, Humboldt University of Berlin, Berlin, Germany*

Saccades are quick, ballistic eye movements characterized by consistent movement patterns: as their amplitude increases, so do their duration (linearly) and peak velocity (exponentially), a consistent relationship referred to as the main sequence. While this relation holds generally true, individual saccades can differ in vigor, i.e., moving faster or slower than expected. Another characteristic defining saccades is that they are preceded by a shift of presaccadic attention from the fixation to the saccade target location.

We designed a novel trial-based monetary reward paradigm to study simultaneously the reward-related modifications of saccade kinematics to random and predictable locations, and the potential impact on presaccadic attention.

A cue indicated one possible saccade target location among others. At various intervals preceding the saccade, a grating was flashed for 25 ms at the saccade target location. Participants saccaded to the target, were informed about the reward they received, and reported the orientation of the grating. The monetary reward was contingent on saccadic velocity. Participants were rewarded for making fast or slow saccades either in different experimental blocks to random locations (Experiment 1 and 2) or to specific fast- or slow-rewarded locations while they were randomly cued from trial to trial (Experiment 3).

In blocks rewarding fast movements, saccades had significantly higher velocity than in blocks rewarding slow movements. Simultaneously, we observed a shift in the time course of presaccadic attention. Rewarding slow saccades revealed a significant disadvantage in performance just before the saccade. Changing the reward rule from trial to trial had no effect on saccadic vigor, nor presaccadic attention even when the rewarded locations were predictable.

Our results demonstrate that the saccadic vigor and the time course of presaccadic attention can be strongly modulated by the reward associated with the movement.

FINANCIAL SUPPORT: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 865715) and the Heisenberg Programme of the Deutsche Forschungsgemeinschaft (grants RO 3579/8-1 and RO 3579/12-1) granted to MR.

## S20.4. OBJECTS OF TRUTH: PREDICTING THE AUTHENTICITY JUDGEMENT OF JOURNALISTIC IMAGES BASED ON ITS CONTENTS, AN EYE-TRACKING STUDY

Laurent Beaupoil, Beata Pacula, Michał Kuniecki

*Institute of Psychology, Jagiellonian University, Kraków, Poland*

In contemporary online journalism, photographs play a central role in convincing users of the veracity of news. This can push forward "fake news", and eclipse real information. Although prior research on textual misinformation shows that directing attention to specific textual elements influences perceived authenticity, comparable research on images remains scarce.

We investigated which types of visual content influence the subjective perception of photographs as real or fake, and how visual attention to these elements affects authenticity judgments.

All stimuli were genuine press photographs obtained from the Polish Press Agency/European Press Agency. A large, representative Polish sample (N=327) provided authenticity judgments along with narrative justifications. Qualitative analysis of these justifications yielded 15 content categories (e.g., Poverty, Protest, Child, Natural Disaster, Medical, Military, Fire, Wounds). Through another study, we defined Regions of Interest (ROIs) in the images. Machine learning classifiers were trained to predict authenticity judgments based on attributed content. A free-viewing eye-tracking study (N=50) examined how visual attention predicted authenticity decisions. Participants were unaware that all photographs were real and that they would later evaluate authenticity. Mixed-effects models were applied using multiple gaze measures.

Classifiers achieved a minimum accuracy of 75%. Feature analysis identified Fire, Protest, and Natural Disaster as the strongest predictors of fake judgments. Eye-tracking analyses showed that sustained attention to Medical and Wounds content increased the likelihood of judging images as real, whereas attention to Child, Military, and Natural Disaster decreased it.

These findings demonstrate that specific visual contents, and sustained attention to them, can shape perceived authenticity and provide a basis for automated systems capable of identifying images likely to be perceived as misleading.

FINANCIAL SUPPORT: This research has been supported by the Polish National Science Centre (grant number 2020/39/B/HS6/03324).

## S21.1. NEURONAL CIRCUITS REGULATING SOCIAL PREFERENCES

Felix Leroy

*Institute of Neurosciences, Spanish National Research Council – Miguel Hernández University, Alicante, Spain*

Social preference, the decision to interact with one conspecific over another, is a feature displayed by gregarious animals that is critical to navigate their social space. Thus, adult rodents prefer to interact with their kin, individuals from specific strains, and members of the opposite sex. In addition to innate factors (e.g., kin, strain, and sex), social preference is also influenced by social memory, social hierarchy, and the affective state of the conspecific. In the lecture, I will explore brain pathways and neuronal mechanisms controlling social preference.

## S21.2. NEUROECONOMICS OF SOCIAL INFLUENCE

Alicja Puścian

*Centre of New Technologies, University of Warsaw, Warsaw, Poland*

Insights from mice tested under semi-naturalistic conditions. Social context is central to how individual preferences emerge. While individual taste preferences are well characterized, the dynamics of group conformity and its persistence after social influence remain poorly understood. We investigated how changing social contexts affect taste preferences in mice and examined the underlying neural mechanisms.Voluntary behavior of group-housed mice was assessed using the automated Eco-HAB assay, which closely follows murine ethology. Individual taste preferences were first measured during brief, isolated access to two nutritionally equivalent flavors. The social environment was then modified to allow group access to the same rewards, enabling mutual influence, followed by a return to isolated testing. Group housing induced convergence toward a shared preference, which reversed once mice were tested individually, indicating that social influence is strong but transient. Chemogenetic activation of parvalbumin-expressing neurons in the prelimbic cortex reduced sociability, potentially disrupting the transmission of social influence – a mechanism currently under investigation.

## S21.3. OLFACTORY BULB ASTROCYTES LINK SOCIAL TRANSMISSION OF STRESS TO COGNITIVE ADAPTATION IN MALE MICE

Paula Gómez-Sotres[1,2], Urszula Skupio[1], Tommaso Dalla-Tor[1], Doriane Gisquet[1], Astrid Cannich[1], Itziar Bonilla-Del Rio[3], Nagore Puente[3], Pedro Grandes[3], Arnau Busquets-Garcia[2], Jaideep S. Bains[4], Giovanni Marsicano[1]

[1] *U1215 Neurocentre Magendie, Bordeaux, France*

[2] *IMIM-Hospital del Mar Medical Research Institute, Parc de Recerca Biomèdica de Barcelona, Barcelona, Spain*

[3] *Department of Neurosciences, Faculty of Medicine and Nursing, University of the Basque Country, Leioa, Spain*

[4] *Krembil Brain Institute, University Health Network, Toronto, Canada*

Emotions and behavior can be affected by social chemosignals from conspecifics. For instance, olfactory signals from stressed individuals induce stress-like physiological and synaptic changes in naïve partners. Direct stress is also known to alter cognition; however, the impact of socially transmitted stress on memory processes remains unknown.

Our research shows that exposure to chemosignals produced by stressed individuals is sufficient to impair memory retrieval in unstressed male mice. This effect requires astrocytic control of olfactory bulb information processing mediated by mitochondria-associated CB1 receptors (mtCB1). Using targeted genetic manipulations, in vivo $Ca^{2+}$ imaging, and behavioral analyses, we demonstrate that mtCB1-dependent regulation of mitochondrial $Ca^{2+}$ dynamics is necessary for processing olfactory information from stressed partners and determining its cognitive consequences.

These findings identify olfactory bulb astrocytes as a critical link between social odors and their behavioral meaning.

## S21.4. RETHINKING DYNORPHIN IN SOCIAL BEHAVIOUR

Klaudia Misiołek

*Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

The endogenous opioid system shapes the rewarding effects of social interaction in adolescent mice. This led us to hypothesize that the opioid system itself undergoes developmental reorganization. To explore this, we analysed the expression of endogenous opioid peptide and receptor genes in the prefrontal cortex and striatum across early, mid and late adolescence (P28, P38, P44). We found that these changes were not global, but selective to the dynorphin/KOR system, with region- and cell-type-specific alterations. To determine whether this reorganization influences social reward, we disrupted dynorphin/KOR signalling, either pharmacologically or in Pdyn knockout mice, and observed reduced social conditioned place preference (CPP) specifically in late adolescence. In adulthood, overall social CPP was lower than in late adolescence, and Pdyn knockout had no detectable effect on CPP. Under baseline (no stress) conditions, dynorphin removal altered other aspects of social behaviour, improving social memory without affecting anxiety-like behaviour. In contrast,

under stress, dynorphin signalling had a broader effect on reward processing, as pharmacological KOR blockade prevented stress-induced reductions in both social and cocaine CPP. Together, these findings show that dynorphin does not simply mediate stress or aversion, but contributes to the developmental organization and context-dependent expression of social behaviour.

## S22.1. OXYSTEROL-DRIVEN MODULATION OF LYMPHOCYTE DYNAMICS MIMICS KEY MECHANISMS OF ACTION OF LEADING THERAPIES FOR MULTIPLE SCLEROSIS

Aleksandra Rutkowska

*Medical University of Gdańsk, Gdańsk, Poland*

Oxysterols acting through the EBI2 (GPR183) receptor pathway are emerging as regulators of immune cell trafficking and CNS repair. We investigated the main endogenous EBI2 ligand, oxysterol 7α,25-OHC, and found that its administration markedly reduced circulating lymphocytes while at the same time increasing their accumulation in the spleen, without inducing cell toxicity. This pattern of lymphocyte redistribution resembles key mechanisms shared by several high-efficacy disease-modifying therapies (DMTs) for multiple sclerosis (MS), suggesting that targeting EBI2–oxysterol signalling may represent a therapeutically relevant immunomodulatory axis. Complementary analysis of cerebrospinal fluid and serum from MS patients provide further support for the involvement of oxysterols in the pathophysiology of MS. Together, these findings identify oxysterol-mediated modulation of lymphocyte dynamics as a safe and promising immunoregulatory mechanism with potential relevance for MS and other immune-mediated conditions. Ongoing analyses in human samples aim to validate these results and further define their applicability to therapeutic development.

## S22.2. MOLECULAR CROSSTALK BETWEEN EBI2 AND LXR SIGNALING AND THEIR EFFECT ON NEUROIMMUNE REGULATION AND MYELINATION

Fatimah, Aleksandra Rutkowska

*Department of Anatomy and Neurobiology, Medical University of Gdańsk, Gdańsk, Poland*

7α,25-dihydroxycholesterol (7α,25OHC) is an oxysterol and agonist of Epstein Barr virus-induced gene 2 (EBI2; GPR183), a receptor that regulates innate and adaptive immune responses in the periphery and CNS. 7α,25OHC accelerates remyelination in the cuprizone model and upregulates the synthesis of multiple lipid classes. Oxysterols also activate liver X receptors (LXRs), which modulate cholesterol metabolism and inflammatory signaling.

To investigate whether 7α,25OHC influences LXR pathways, organotypic cerebellar slices from wild type and EBI2 knockout mice were demyelinated and treated with 7α,25OHC, with or without LXR antagonists.

Exogenous 7α,25OHC increased LXRβ expression and regulated canonical LXR targets, including ABCA1 and SREBP1c, even in EBI2-deficient slices, indicating EBI2 independent signaling.

These findings suggest that 7α,25OHC reshapes oxysterol metabolism and broader signaling networks in the CNS, highlighting its potential as a modulator of lipid-mediated pathways relevant for remyelination and neuroinflammation.

FINANCIAL SUPPORT: The project received funding from the National Science Centre, Poland, grant registration number: 2022/47/D/NZ3/02613 (AR).

## S22.3. MECHANOSENSITIVE PIEZO ION CHANNELS AND AMYLOID BETA PATHOLOGY IN THE REGULATION OF OLIGODENDROCYTE FUNCTION AND MYELINATION

Piotr Pobiarzyn, Aleksandra Rutkowska

*Department of Anatomy, Division of Anatomy and Neurobiology, Medical University of Gdańsk, Gdańsk, Poland*

Piezo channels are mechanosensitive ion channels that translate mechanical stimuli into intracellular signaling and are enriched in white-matter regions. Evidence indicates that Piezo1/2 may regulate oligodendrocyte maturation and myelin stability: inhibition appears protective in demyelination models, while activation is linked to myelin disruption. Alzheimer's disease also involves marked white-matter pathology, and both OPCs and mature oligodendrocytes are vulnerable to amyloid beta (Aβ). These observations suggest that

Piezo signaling and Aβ-related stress may converge on pathways controlling myelination, potentially contributing to white-matter degeneration.

This project will examine how Piezo channels influence OPC differentiation and myelin formation and whether Aβ alters these processes through disrupted mechanotransduction. It further aims to identify shared molecular mechanisms and test whether Piezo modulation can preserve oligodendrocyte function under AD-relevant conditions

Primary OPCs and cerebellar slices will be studied under controlled mechanical conditions with pharmacological or genetic modulation of Piezo activity, alongside exposure to oligomeric and fibrillar Aβ. Responses will be evaluated using calcium imaging, signaling assays, and myelin-marker analysis, with pathway-focused transcriptional profiling. Functional relevance will be assessed in neuron–glia co-cultures to determine effects on myelination.

Piezo activation is expected to impair OPC maturation, whereas inhibition may promote differentiation. Aβ will likely exacerbate mechanosensitive stress signaling, revealing overlapping pathways related to calcium dynamics and cellular structure. Targeting Piezo could partially rescue myelination deficits.

Defining the interaction between Piezo signaling and Aβ toxicity may clarify mechanisms underlying white-matter vulnerability in Alzheimer's disease and highlight Piezo channels as potential targets for protecting oligodendrocyte function.

## S22.4. AAV-MEDIATED DELIVERY OF A PROLIFERATION-INDUCING LIGAND (APRIL) TO CORTICAL NEURONS LIMITS INFLAMMATION AND DEMYELINATION IN THE CORPUS CALLOSUM OF THE CUPRIZONE MOUSE MODE

Leonardo Ricciardi[1,2,5], Federica Di Marco[3,4], Marion Decrop[1,2], Julia Di Stefano[1,2,5], Tejaswini Kakunuri[6], Joëlle van Rijswijk, Nicholas Vidas-Guscic[1,2,1,2], Yousra El Ouaamari[5], Alexandro A. Bufi[5,7], Ignace Van Spilbeek, Johan Van Audekerke[1,2,1,2], Carole Faghel Esther Bartholomeus[1,2], Eva Lion Nathalie Cools[5,5], Steven Van Laere[8], Ilaria Cicalini[3,4], Vincenzo De Laurenzi[3,4], Una FitzGerald[9,10], Damiana Pieragostino[3,4], Bertrand Huard[6], Piero Del Boccio[3,4,11], Peter Ponsaerts[5], Marleen Verhoye[1,2]

*[1] Bio-Imaging Lab, University of Antwerp, Wilrijk, Belgium*

*[2] μNEURO Research Centre of Excellence, University of Antwerp, Wilrijk, Belgium*

*[3] Center for Advanced Studies and Technology (CAST), G. d'Annunzio University of Chieti-Pescara, Chieti, Italy*

*[4] Department of Innovative Technologies in Medicine and Dentistry, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy*

*[5] Laboratory of Experimental Hematology, Vaccine and Infectious Disease Institute (Vaxinfectio), University of Antwerp, Wilrijk, Belgium*

*[6] TIMC-IMAG Laboratory, University Grenoble Alpes, La Tronche, France*

*[7] Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy*

*[8] Center for Oncological Research (CORE), Integrated Personalized & Precision Oncology Network (IPPON), University of Antwerp, Antwerp, Belgium*

*[9] CÚRAM, Centre for Research in Medical Devices, Galway Neuroscience Centre, Biomedical Engineering, University of Galway, Galway, Ireland*

*[10] Galway Neuroscience Centre, University of Galway, Galway, Ireland*

*[11] Department of Pharmacy, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy*

A Proliferation-Inducing Ligand (APRIL) is a tumour necrosis factor superfamily member with multiple effector roles in the peripheral and central nervous system (CNS). In the CNS, APRIL helps maintain immune homeostasis and supports neuronal survival, yet whether it can therapeutically modulate acute neuro-inflammatory and neurodegenerative processes is unclear.

To investigate this in a severe neuro-inflammatory context, we tested whether local APRIL delivery can influence microglial/astrocytic responses and demyelination in the cuprizone (CPZ) mouse model of neuro-inflammation and demyelination.

We applied adeno-associated virus (AAV)–mediated gene transfer to drive APRIL expression by cortical neurons positioned directly above the splenium of the corpus callosum. Following CPZ intoxication, we evaluated microglial and astrocytic activation and myelin content by in vivo T2 and diffusion magnetic resonance imaging (MRI) and validated them by post-mortem quantitative immunohistochemistry (IHC) and bulk transcriptomic and proteomic profiling.

T2 and diffusion MRI, corroborated by quantitative IHC, demonstrated that secretion of APRIL by cortical neurons attenuated CPZ-induced microglial recruitment and demyelination in the splenium, but not in the cortex. Integrated transcriptome-proteome analysis of splenium and cortex, further supported by IHC, highlighted astroglial lipid-metabolic circuitry – marked by the astrocytic fatty-acid-binding protein 7 (Fabp7) – as a candidate axis underlying APRIL's effects to reduce inflammatory cell recruitment and myelin preservation.

AAV-mediated expression of APRIL by cortical neurons counteracted microglial recruitment and demyelination in the splenium of CPZ-treated mice. Together, our findings provide a framework for the mechanistic

dissection of APRIL's therapeutic potential and further translational evaluation in CNS pathologies characterised by acute neuro-inflammatory responses.

FINANCIAL SUPPORT: This research was funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813263 (PMSMatTrain). Additional support was provided by the Fund for Scientific Research Flanders (FWO-Vlaanderen) of the Flemish Government (FWO sabbatical bench fee K800224N) and the 'Bijzonder Onderzoeksfonds' (BOF) of the University of Antwerp (UA BOF-GOA 2020, UA BOF-SEP 2022 and BOF Research Sabbatical 2023).

## S22.5. DISTINCT TRANSCRIPTIONAL SIGNATURES OF MOTONEURON FATE IN RESPONSE TO NEONATAL NERVE REGENERATION OR DEGENERATION

Olga Blauth[1], Agnieszka Misztak[2], Beata Kucharz[1], Urszula Ławrynowicz[2], Krzysztof Miazga[1], Urszula Sławińska[1], Jakub Mieczkowski[2], Małgorzata Zawadzka[1]

*[1] Laboratory of Neuromuscular Plasticity, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*
*[2] 3P Medicine Laboratory, International Research Agenda, Medical University of Gdańsk, Gdańsk, Poland*

Peripheral nerve injuries during early postnatal life frequently result in limited axonal regrowth and extensive motoneuron degeneration, in contrast to the regenerative capacity observed in adults. Understanding the cellular mechanisms underlying this developmental difference is critical for improving neonatal nerve repair strategies.

To identify spinal cord cellular responses and transcriptional programs that distinguish successful regeneration from regenerative failure following neonatal peripheral nerve injury.

We employed a sciatic nerve grafting model in newborn rats, in which an older donor nerve fragment (P6) was transplanted into a younger recipient (P3), thereby leveraging the donor tissue's more advanced developmental state to enhance regeneration. In contrast, grafts from age-matched donors (P3/P3) fail to support effective regrowth. We also applied single-nucleus RNA sequencing (snRNA-seq) together with immunohistochemistry to define injury-driven shifts in spinal cord cell populations and transcriptional responses.

Given the absence of robust neonatal motoneuron markers, this approach provided an unbiased framework to resolve cell-type-specific injury programs across the spinal cord. Enhanced regeneration was associated with increased survival of ChAT$^+$ motoneurons, whereas failed regeneration correlated with motoneuron degeneration. snRNA-seq revealed distinct injury-responsive transcriptional states across multiple cell types. Notably, regenerative conditions exhibited an early increase in Iba1$^+$ microglial cells, whereas degenerative conditions showed delayed and prolonged microglial activation.

Together, these findings suggest that injury-induced microenvironmental cues and neuroimmune dynamics critically shape regenerative capacity and motoneuron survival following neonatal peripheral nerve injury.

FINANCIAL SUPPORT: Funding: National Science Centre, Poland (NCN 2020/37/B/NZ4/04065).

## S23.1. COLD-WATER IMMERSION AS A MODEL OF HUMAN ADAPTATION: INSIGHTS FROM BRAIN, BEHAVIOUR, AND AFFECT

Ala Yankouskaya

*Bournemouth University, Bournemouth, United Kingdom*

Acute physiological strain offers a uniquely tractable model for understanding how the human brain reorganises under pressure.

In this talk, I will present converging evidence from neuroimaging, electrophysiology, and large-scale behavioural data examining how brief cold-water immersion (CWI) influences affective state and neural function.

First, resting-state fMRI demonstrates that even a 5-minute immersion triggers rapid reconfiguration of large-scale networks supporting self-referential processing, salience detection, and attentional control, with changes in connectivity closely paralleling shifts in positive and negative affect. New EEG findings extend this picture, showing immediate reductions in frontal alpha power and increased theta–beta coupling following CWI, biomarkers consistent with elevated vigilance, cognitive readiness, and improved mood regulation. Complementing the laboratory work, a large patient survey (N=700) reveals that individuals who regularly engage in cold-water practices report lower depressive symptoms, greater perceived stress resilience, and stronger beliefs in their ability to regulate

mood, highlighting the broader psychosocial relevance of these physiological effects.

Together, these data suggest that acute cold exposure engages a multi-level adaptive response, spanning brain networks, electrophysiological dynamics, and subjective mood, that may help explain its emerging therapeutic potential.

## S23.2. THE FUNCTION OF THE ANTERIOR CINGULATE CORTEX UNDER COGNITIVE STRAIN

Batuhan Serif Cakir, Gizem Arabaci, Ben Parris

*School of Psychology, Bournemouth University, Bournemouth, England*

The anterior cingulate cortex (ACC) is central to monitoring control demands, signalling when situations generate cognitive strain, in the form of conflict or uncertainty. Based on the conflict monitoring account, the ACC detects response conflict when competing actions are activated, whereas task conflict theory argues that the ACC monitors competition between broader task sets.

By directly testing these competing predictions using a Stroop task, this work aims to clarify the ACC's functional role.

Using fMRI, participants' performance is measured across mixed blocks (containing equal proportions of congruent, incongruent, neutral, and letter-string trials, prompting high unpredictability) and pure blocks (each containing only one trial type; reducing cognitive demands).

Comparing ACC activity between pure versus mixed blocks, and across congruent, incongruent, neutral, and letter-string trials, allows a direct test of whether the ACC is activated primarily by response conflict, or by task-set conflict and unpredictability.

By outlining when and how the ACC is engaged, this talk will advance current discussions on its role in cognitive control and monitoring, contributing to ongoing debates about its function.

## S23.3. HIPPOCAMPAL TOTAL, SUBREGION, AND SUBFIELD VOLUME ALTERATIONS IN LATE-LIFE DEPRESSION

Brianca Renfro[1], Sarah M. Szymkowicz[1], Ke Xu[2], Kimberly Albert[1], LaShonda Price[1], Hakmook Kang[2], Damian Elson[1], John Patrick Begnoche[1], Brian D. Boyd[1], Helmet T. Karim[3,4], Bennett A. Landman[5], Olusola A. Ajilore, MD[6], Carmen Andreescu[3], Warren D. Taylor[1,7]

[1] *Center for Cognitive Medicine, Department of Psychiatry and Behavioral Science, Vanderbilt University Medical Center, Nashville, United States*

[2] *Department of Biostatistics, Vanderbilt University Medical Center, Nashville, United States*

[3] *Department of Psychiatry, University of Pittsburgh, Pittsburgh, United States*

[4] *Department of Bioengineering, University of Pittsburgh, Pittsburgh, United States*

[5] *Departments of Computer Science, Electrical Engineering, and Biomedical Engineering, Vanderbilt University; Department of Radiology and Radiological Sciences, Vanderbilt University Medical Center, Nashville, United States*

[6] *Department of Psychiatry, University of Illinois Chicago, Chicago, United States*

[7] *Geriatric Research, Education, and Clinical Center, Veterans Affairs Tennessee Valley Health System, Nashville, United States*

Despite consistent support for smaller total hippocampal volumes in late-life depression (LLD), there is less consensus on LLD's relationship with hippocampal subregions and subfields. This is particularly germane for interactive effects of aging in context of depression, as individuals with LLD may exhibit accelerated aging processes.

To investigate hippocampal total, subregion, and subfield volumes in LLD versus non-depressed controls and how age moderates these effects.

We examined 260 depressed and 140 non-depressed older adults who completed 3T MRI and extracted hippocampal subregion and subfield volumes. Primary analyses investigated effects of LLD diagnosis, active *vs.* remitted depression, and age of onset on hippocampal volumes. Secondary analyses evaluated interactive effects of age and these diagnostic groupings on hippocampal volumes.

Compared to controls, the LLD group exhibited smaller total hippocampal volumes, with smaller volumes of the bilateral hippocampal head and the right hemisphere body, tail, CA1, and molecular layer. When comparing current and remitted LLD, individuals with current LLD exhibited smaller right total hippocampus, head, body, and molecular layer volumes compared to controls. They also exhibited smaller CA1, CA2, and CA4/dentate gyrus volumes compared to both control and remitted LLD groups. No regions differed between control and remitted LLD groups. We did not observe significant group differences in any volume measure between LLD individuals with an early- or later-life depression onset. There was no significant age by di-

agnostic group interactions on hippocampal volumes across any analyses.

This cross-sectional work supports diagnostic, but not accelerated aging, effects of depression on hippocampus volumes. In contrast to recently remitted LLD, currently depressed individuals exhibited smaller volumes primarily in the right hippocampus.

FINANCIAL SUPPORT: This work was supported by National of Institute of Health grants R01 MH102246, R21 MH099218, R01 MH123662, R61 MH122464, R01 MH121619, R01 MH121620, and R01 MH121384, and the National Center for Advancing Translational Sciences grants UL1 TR000445, UL1 TR002243, and KL2 TR002245.

## S23.4. LONG-TERM EXPOSURE TO AIR POLLUTION IMPACTS ACTIVITY IN BRAIN REGIONS INVOLVED IN INHIBITORY CONTROL IN 10- TO 13-YEAR-OLD CHILDREN

Mikołaj Compa[1,4], Yarema Mysak[2], Clemens Baumbach[3], Paulina Lewandowska[4], Aleksandra Domagalik-Pittner[5], Bartosz Kossowski[6], Katarzyna Kaczmarek-Majer[7], Anna Degórska[7], Krzysztof Skotak[7], Katarzyna Sitnik-Warchulska[9], Małgorzata Lipowska[8], Bernadetta Izydorczyk[1], James Grellier[2], Iana Markevych[1], Marcin Szwed[1]

[1] *Institute of Psychology, Jagiellonian University, Kraków, Poland*

[2] *European Centre for Environment and Human Health, University of Exeter Medical School, Penryn, United Kingdom*

[3] *Institute and Clinic for Occupational, Social and Environmental Medicine, LMU University Hospital, LMU Munich, Munich, Germany*

[4] *Faculty of Psychology, SWPS University of Social Sciences and Humanities, Kraków, Poland*

[5] *Centre for Brain Research, Jagiellonian University, Kraków, Poland*

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

[7] *Institute of Environmental Protection-National Research Institute, Warsaw, Poland*

[8] *Institute of Psychology, University of Gdańsk, Gdańsk, Poland*

[9] *Institute of Applied Psychology, Faculty of Management and Social Communication, Jagiellonian University, Kraków, Poland*

The development of inhibitory control, a core component of cognitive control, can be influenced by environmental factors. We investigated whether exposure to particulate matter with diameter≤10 μm (PM10) and nitrogen dioxide (NO2) across different life-time periods is related to the neural correlates of inhibitory control in 10- to 13-year-old children from southern Poland.

To investigate whether long-term exposure to air pollution is associated with task-related brain activity during inhibitory control, and to examine whether these associations differ between individuals with ADHD and typically developing peers.

Task functional magnetic resonance imaging (task fMRI) measures brain activity while participants perform specific cognitive or behavioral tasks. We investigated inhibition using a Go/NoGo task during task fMRI and tested associations between neural correlates of inhibitory control and exposure to air pollution during prenatal, early-life, and current life periods. The study population comprised children from the NeuroSmog study with Attention-Deficit/Hyperactivity Disorder (ADHD, n=143) and their typically developing peers (n=385).

Higher current exposure to PM10 was significantly associated with reduced brain activation during response inhibition in key cognitive control networks, including the dorsolateral prefrontal cortex and anterior cingulate cortex. We did not observe significant interactions between our participants' ADHD diagnosis and their exposure to air pollution

Long-term exposure to air pollution was associated with impairments in brain function related to inhibition in both ADHD children and their typically developing peers. Our findings add novel, pertinent evidence to the growing body of research indicating that air pollution negatively impacts the development of executive function in children and suggests that the same mechanisms that underlie pollution's effects on the brain may also lead to the increased incidence of ADHD.

FINANCIAL SUPPORT: Supported by the "NeuroSmog: Determining the impact of air pollution on the developing brain" (Nr. POIR.04.04.00-1763/18-00) grant implemented as part of the TEAM-NET programme of the Foundation for Polish Science, co-financed from EU resources obtained from the European Regional Development Fund under the Smart Growth Operational Programme, also by a grant from the Priority Research Area ("Anthropocene") under the Strategic Programme Excellence Initiative at Jagiellonian University, and by a grant from the National Science Centre, Poland (grant no. K/NCN/000514), all of which were obtained by Marcin Szwed.

## S23.5. VISUAL SYSTEM VULNERABILITY TO ACUTE HYPOXIA: STRUCTURAL REMODELLING AND PHARMACOLOGICAL MODULATION

Ruzanna A. Shushanyan, Anna V. Grigoryan, Tamara A. Abgaryan, Anna F. Karapetyan

*Department of Human and Animal Morphology and Physiology, Research Institute of Biology, Yerevan State University, Yerevan, Armenia*

Acute exposure to hypoxic environments, such as high altitude, can lead to transient or lasting visual disturbances, including high-altitude retinopathy (HAR). These conditions arise from disrupted neurovascular regulation and impaired oxygen delivery to neural tissues. The retina is highly vulnerable to hypoxia, as is the visual cortex, making the visual system a sensitive readout of hypoxia-induced neurodegeneration.

In this study, we used a rat model of acute hypobaric hypoxia to examine how oxygen deprivation alters retinal cytoarchitecture and neurovascular integrity, and whether corticosteroid intervention can modify these effects.

Animals were exposed to a simulated high altitude environment, with a subset receiving dexamethasone pretreatment. Hypoxic injury was induced in a hypobaric chamber ($FiO_2$=16.6%) for 6 hours/day over 3 days. Animals in the treatment group received a single intraperitoneal dose of dexamethasone (1 mg/kg) before hypoxic exposure. Histomorphological and ultrastructural analyses have been utilized to reveal hypoxia-associated cellular injury and the modulatory effect of the corticosteriod pretreatment.

Hypoxic exposure resulted in marked structural remodeling of the inner retina, including thickening of the ganglion cell and nerve fiber layers, vascular leakage, and neuronal swelling, accompanied by degenerative changes in the visual cortex. In contrast, dexamethasone pretreatment attenuated inflammatory responses, preserved barrier integrity, and maintained retinal and cortical neuronal organization.

These findings demonstrate that acute hypoxia rapidly reshapes retinal structure through inflammatory and neurovascular mechanisms, and that pharmacological modulation can partially restore neural integrity. HAR may therefore provide a useful experimental framework for studying hypoxia-driven neurodegeneration across the visual system.

FINANCIAL SUPPORT: The work was supported by the Higher Education and Science Committee of MESCS RA (Research project No. 25RG-1F181).

## S24.1. STRESS AND NEUROPHARMACOLOGY IN THE PLACENTA-BRAIN AXIS

Cristiana Cruceanu

*Department of Physiology and Pharmacology, Karolinska Institutet, Solna, Sweden*

Dr. Cruceanu will discuss the work of the Developmental and Translational Neurobiology research group, which she leads at the Karolinska Institutet in Stockholm, Sweden.

Motivated by the developmental origins of health and disease hypothesis, she will show data aiming to elucidate how prenatal environment exposures can shape fetal neurodevelopment.

State-of-the-art approaches using longitudinal human cohorts, population health register data, and in-vitro and ex-vivo model systems and single-cell multi-omics, will investigate the molecular responses and implications of exposure to stress hormones and synthetic glucocorticoids as well as psychotropic medications, especially selective serotonin reuptake inhibitors.

Converging lines of evidence will concentrate on the effect of these exposures in the most important human tissues along the maternal-fetal interface: the brain and the placenta.

Important implications of studying molecular mechanisms in human-specific models will be discussed.

FINANCIAL SUPPORT: The work presented has been supported by Karolinska Institutet, the Swedish Research Council (Vetenskapsrådet), the Brain and Behavior Research Foundation (BBRF), and the Swedish Society for Medical Research (SSMF).

## S24.2. DECODING STRESS HORMONE SIGNALING IN THE HUMAN BRAIN USING IPSC-DERIVED NEURAL CELLS

Viorica Raluca Conțu[1], Aleksandra Herud[1], Mateusz Zięba[2], Marcin Piechota[2], Michał Korostyński[2], Michał Ślęzak[1]

[1] *Biology of Astrocytes Research Group, Life Science and Biotechnology Center, Łukasiewicz Research Network – PORT Polish Center for Technology Development, Wrocław, Poland*

[2] *Laboratory of Pharmacogenomics, Department of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

Stress is a major risk factor for a wide range of psychiatric disorders, yet the molecular mechanisms through which stress hormones affect the human brain remain incompletely understood. Glucocorticoids (GCs) mediate the stress response primarily through the glucocorticoid receptor (GR), a transcription factor that operates in a tissue- and cell-specific manner. While GR-regulated pathways are well characterized in peripheral organs, the effects of GCs in brain cells are less clear.

Here, we investigated GR signaling in a human-relevant system using surface-attached 3D mixed neural cultures derived from iPSCs.

Cultures were exposed to cortisol or the synthetic GR agonist dexamethasone, followed by whole-transcriptome sequencing in addition to targeted analyses using immunocytochemistry and qPCR.

Our results reveal robust activation of canonical GR bona fide genes across neural populations, alongside distinct cell-type- and treatment-specific components of the response. These findings highlight heterogeneity in GR signaling within human neural systems and suggest that differential transcriptional responses may contribute to stress-related vulnerability or resilience.

Overall, this work provides insight into how stress hormones shape molecular programs in the human brain and offers a framework for studying mechanisms relevant to stress-related psychiatric disorders and therapeutic target discovery.

FINANCIAL SUPPORT: This work was supported by NCN OPUS grant GRtraits nr. 2022/45/B/NZ5/03188.

## S24.3. INVESTIGATION OF AGING-RELEVANT MECHANISMS OF NEURODEGENERATION IN PATIENT-DERIVED MODELS

Karolina Cierluk[1,2], Ewa Mrówczyńska[1], Agnieszka Krzyżosiak[1]

[1] *Mechanisms of Neurodegeneration Group, Łukasiewicz Research Network – PORT Polish Center for Technology Development, Wrocław, Poland*

[2] *Wrocław Medical University, Wrocław, Poland*

Neurodegenerative diseases (NDDs) are devastating, age-associated disorders characterized by progressive neuronal loss that, despite extensive research efforts, remain largely incurable. Impairment of protein quality control (PQC) is a central pathogenic feature of NDDs, promoting the accumulation of misfolded pathogenic proteins and progressive cellular dysfunction. Notably, PQC efficiency declines with age, the strongest risk factor for neurodegeneration.

Translational research in NDDs has proven challenging because many traditional experimental models have failed to accurately recapitulate the complexity of human disease mechanisms, limiting their predictive and translational value. The implementation of cell reprogramming methods has opened a new era in research and enabled modeling NDD in the context of human genetics.

In our research, we use human models derived from patients to study the molecular mechanisms of NDDs. Through the implementation of direct-reprogramming methods, which omit the pluripotent stage, cells retain the transcriptional and epigenetic signatures of the donors cells which allow us to study NDDs mechanisms in the context of aging.

In this aging-relevant environment we investigate the mechanisms of PQC decline and search for modifiers of age-relevant disease phenotypes. The implementation of the neuron-astrocyte co-culture systems enables us to investigate how protein homeostasis in astrocytes contributes to the development of neuronal dysfunctions.

With this translational approach, we hope to reveal aging-relevant molecular mechanisms underlying neurodegenerative pathology both in neurons and astrocytes and discover factors to be further explored for therapeutic purposes in NDDs.

FINANCIAL SUPPORT: The project is financed from the Sonata Bis 11 Grant (2021/42/E/NZ3/00439) – National Science Centre Poland.

## S24.4. STANDARDIZING CRYOPRESERVATION OF VENTRAL FOREBRAIN ORGANOIDS TO STUDY MITOCHONDRIAL REGULATION OF THE EARLY HUMAN NEURODEVELOPMENT

Erkan Metin[1], Wiktoria Pioruńska[2], Zuzanna Kuczyńska[1], Marzena Zychowicz[1], Wioletta Lech[1], Paweł Leszczyński[1], Aleksandra Duchnowska[3], Leonora Bużańska[1]

[1] *Department of Stem Cell Bioengineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland*

[2] *Utrecht University, Utrecht, Netherlands*

[3] *Laboratory of Molecular Basis of Neurodegeneration, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland*

Early human neurodevelopment involves dynamic changes in mitochondrial function. In brain organoid cultures, variability between independent differentiation experiments and batches can make it difficult to compare equivalent developmental stages across different genetic backgrounds. Establishing reliable cryopreservation methods for patient-derived organoids may help preserve defined developmental time points and enable more consistent analysis of mitochondrial changes during early human neurodevelopment.

This study aimed to evaluate different cryopreservation strategies in patient-derived ventral forebrain organoids and to determine how these methods influence early post-thaw recovery and downstream mitochondrial analyses.

Human ventral forebrain organoids were generated from induced pluripotent stem cells, including both non-disease reference lines and Dravet syndrome patient-derived lines. Organoids were cryopreserved using Cryostor and MEDY on day 18 of differentiation and assessed one and two weeks after thawing. Post-thaw recovery was evaluated by measuring organoid size and morphology, examining ventral forebrain progenitor and early neuronal markers using immunocytochemistry, and performing metabolic and viability assays.

We observed clear method-dependent differences in early recovery after cryopreservation. The CryoStor-based method preserved organoid architecture and growth consistency more effectively than the MEDY-based approach, which showed reduced structural stability during early recovery. Ongoing analyses are assessing whether these structural differences are associated with changes in mitochondrial activity and developmental marker expression in both disease and reference organoids.

These findings indicate that standardized cryopreservation improves experimental control by preserving defined developmental stages and enabling more reliable comparisons between disease and reference organoids when investigating early mitochondrial regulation.

FINANCIAL SUPPORT: This study was financed by ABM grant KPOD.07.07-IW.07-0105/25.

## S24.5. UNRAVELING HUNTINGTON'S DISEASE USING IPSC-DERIVED MODELS

Victoria Graffe-Pereira, Magdalena Czeredys

*Huntington's Disease Group, Department of Stem Cell Bioengineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland*

Huntington's Disease (HD) is an inherited motor, cognitive and psychiatric disorder that currently has no cure. Although HD has been primarily addressed as a neurodegenerative disease, alterations in early developmental stages in both adult and juvenile onsets suggest that HD could have a neurodevelopmental origin.

Our aim is to use induced Pluripotent Stem Cells (iPSC) to generate 2D and 3D in vitro models that can serve as platforms to recapitulate neurodevelopment in HD and identify pathological mechanisms behind it.

Transfection of dermal fibroblasts with OCT4, SOX2, KLF4, and C-MYC to generate iPSC lines, differentiation of such lines into 2D neural progenitor cells (NPCs) and medium spiny neurons (MSNs), as well as 3D cortical and striatal organoids. Characterization of iPSC lines and iPSC-derived models through immunofluorescence, PCR and transcriptomic analyses.

We generated iPSC lines from adult and juvenile HD onsets and age-related controls. We used these lines to obtain NPCs, MSNs, and organoids from HD patients and controls. Our models express markers such as Nestin and PAX6 in NPCs, MAP2 and DARPP32 in both MSNs and striatal organoids, as well as SOX2 and TBR1 in cortical organoids, confirming their specific fates. Moreover, we have detected morphological alterations in our HD models that correspond with neurodevelopmental abnormalities in HD previously reported in the literature.

Our iPSC lines have allowed us to generate in vitro models of HD that reproduce pathological features as they grow, potentially reinforcing the hypothesis of an impaired neurodevelopment in HD. Such changes should be further explored and targeted via neuromodulatory compounds, to assess the chances of reversing the obtained phenotypes into a physiological state.

FINANCIAL SUPPORT: NCN Opus 25 grant no. 2023/49/B/NZ3/04131 to MCz.

## S25.1. PSYCHEDELICS AS MODULATORS OF NEUROIMMUNE FUNCTION

Danilo De Gregorio

*Neuropsychopharmacology Unit, Division of Neuroscience, IRCCS San Raffaele Hospital, Milan, Italy*

Psychedelic compounds such as lysergic acid diethylamide (LSD) are gaining renewed interest as potential therapeutics in psychiatry. While their effects on neuroplasticity are well recognized, emerging evidence suggests that they also modulate immune function. Psychedelics acting at 5-HT2A receptors can regulate inflammatory responses, including the inhibition of pro-inflammatory cytokines and the modulation of immune cell activity . Recent studies further highlight the importance of neuroimmune interactions in shaping brain function and behavior . In this talk, I will provide an overview of the immunomodulatory properties of psychedelics and discuss how these compounds may influence neuroimmune crosstalk. Preliminary preclinical observations will be presented to illustrate how psychedelics can impact microglial function, suggesting a role beyond classical anti-inflammatory effects, potentially linked to synaptic plasticity.

## S25.2. INFECTION-SPECIFIC REPROGRAMMING OF MICROGLIA IN ALZHEIMER'S DISEASE

Marta Kamińska[1], Anne Klapper[2], Noemie Dudzinska[1], Stefanie Geissler[2], Nadine Taudte[2], Sebastian Greiser[3], Jan Potempa[4], Holger Cynis[2], Piotr Mydel[1,4]

[1] *Broegelmann Research Laboratory, Department of Clinical Science, Faculty of Medicine, University of Bergen, Bergen, Norway*

[2] *Department of Molecular Drug Design and Target Validation, Fraunhofer Institute for Cell Therapy and Immunology, Halle (Saale), Germany*

[3] *Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany*

[4] *Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland*

Alzheimer's disease (AD) is increasingly recognized as a disorder driven not only by protein aggregation but also by chronic neuroinflammation. While microglia are key regulators of this immune response, their heterogeneity – and how it is shaped by peripheral inflammatory triggers – remains incompletely understood.

Although oral pathogens have emerged as potential upstream contributors to neurodegeneration, their precise influence on microglial phenotypes has yet to be defined.

I will present a study in which we used a dual approach combining in vitro high-dimensional single-cell mass cytometry with in vivo imaging mass cytometry in a hTau-knock in 5xFAD mouse model to dissect how two major periodontopathogens – Porphyromonas gingivalis (Pg) and Tannerella forsythia (Tf) – reprogram microglial subpopulations.

We show that Pg, through its gingipains, drives transient but robust proinflammatory polarization of microglia, eroding regulatory subsets critical for lesion control, while Tf induces a more suppressive and potentially exhausted microglial profile. Importantly, these infection-specific phenotypes are recapitulated in vivo, where Pg infection causes depletion of mixed-activation microglia in the cortex and reshapes spatial interactions with amyloid/pTau-rich lesions.

This demonstrates that virulence factor–dependent reprogramming of microglia contributes to AD-relevant pathology in a region- and lesion-specific manner.

FINANCIAL SUPPORT: This project has been supported by the EU Join Programme ERA-NET JPND/JP-co-fuND2 – Neurodegenerative Disease Research (Gums and Brains project awarded to JP, PM, and HC), the Polish National Science Centre (2019/01/Y/NZ1/00016 to JP), Research Council of Norway (296129 awarded to PM) and Norwegian Health Association, Norway (nr 35318 awarded to MK). MK was supported by the Meltzer Foundation, Norway, and MK, ND and PM were supported by the Broegelmann Research Foundation, Norway. Generation of tau-KI mice was supported by the Alzheimer's Association (grant: 2016-NIRG-396-583).

## S25.3. TYPE I INTERFERON SIGNALING SHAPES MICROGLIAL ACTIVATION AFTER ISCHEMIC BRAIN INJURY

Julia Jarco[1,2], Alba Simats[2,4], Natalia Małek[1], Marta Kamińska[3], Anna Planas[2,4]

[1] *Department of Chemical Biology and Bioimaging, Wrocław University of Science and Technology, Wrocław, Poland*

[2] *Department of Neuroscience and Experimental Therapeutics, Institute for Biomedical Research of Barcelona, Spanish National Research Council, Barcelona, Spain*

[3] *Department of Clinical Science, University of Bergen, Bergen, Norway*

[4] *Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain*

Ischemic stroke triggers a strong neuroinflammatory response involving resident microglia and infiltrating immune cells. Increasing evidence indicates that type I interferon (IFN-I)–related signaling pathways are activated in microglia following brain injury; however, the functional role of this response in shaping neuroimmune and immunometabolic processes remains unclear.

The aim of this study is to investigate the role of type I IFN signaling in microglial activation and immunometabolic responses following ischemic stroke, with particular focus on microglia–immune cell interactions within intact brain tissue.

A mouse model of ischemic stroke was used, including genetically modified mice with ablation of the type I IFN receptor (IFNAR-deficient mice). Immune cells were isolated from brain tissue and analyzed using imaging mass cytometry and flow cytometry to characterize microglial activation states and spatial interactions with infiltrating immune populations at single-cell resolution.

Preliminary analyses indicate that ischemic brain injury is associated with distinct microglial activation patterns linked to IFN-I signaling. Spatial profiling reveals heterogeneous microglial states and region-specific interactions with infiltrating immune cells, suggesting a potential role for IFN-I pathways in shaping the post-stroke neuroimmune microenvironment.

These findings provide insight into the involvement of type I IFN signaling in microglial immunometabolic responses after ischemic stroke. Spatially resolved approaches enable the identification of neuroimmune features that may be inaccessible to dissociative methods and contribute to a better understanding of post-injury neuroinflammation.

FINANCIAL SUPPORT: This work was supported by MCIN/AEI/10.13039/501100011033 (grant PID2023-150949OB-I00) and by the National Science Centre (NCN), Poland (grants 2023/49/B/NZ7/02172 and 2023/05/Y/NZ4/00152).

## S25.4. BEYOND THE BRAIN: SYSTEMIC IMMUNE AND CARDIAC CONSEQUENCES OF STROKE

Alba Simats[1,2]

[1] *Institute of Biomedical Research of Barcelona, Spanish National Research Council, Barcelona, Spain*

[2] *Fundació Clínic per la Recerca Biomèdica – Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain*

Immune responses play a central role in stroke pathology and recovery, with consequences that extend beyond neurological outcome. Rather than being limited to resident microglial responses, post-stroke inflammation reflects a brain–systemic immune axis in which peripheral immune cells and long-term systemic immune alterations also play a central role.

In this talk, I will present our recent work published in Cell, demonstrating that acute brain injury such as ischemic stroke further induces persistent epigenetic reprogramming of circulating innate immune cells, imprinting a peripheral innate memory state that might further compromise patient's outcomes.

Using experimental stoke models and patient samples, we show that this transcriptional rewiring substantially increases susceptibility to secondary complications beyond neurological consequences, such as cardiac dysfunction.

Together, these findings position stroke as a systemic immunological disease and identify immune pathways as promising targets to improve long-term outcomes, acting in complement to immune mechanisms operating within the brain itself.

FINANCIAL SUPPORT: Please see publication Simats et al., Innate immune memory after brain injury drives inflammatory cardiac dysfunction, CELL 2024 Aug 22;187(17): 4637-4655.e26. https://doi.org/10.1016/j.cell.2024.06.028.

## S25.5. CHARACTERISATION OF TAU PATHOLOGY DIFFERENCES BETWEEN BRAIN REGIONS IN SECONDARY AND PRIMARY TAUOPATHIES USING QUANTITATIVE MASS SPECTROMETRY

Diana Piotrowska[1], Elena Camporesi[1], Malin Wennström[2], Divya Bali[3], Nklas Mattsson-Carlgren[3], Laia Montoliu-Gaya[1], Johan Gobom[1], Sebastian Palmqvist[3], Henrik Zetterberg[1], Oskar Hansson[3], Gunnar Brinkmalm[1]

*[1] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Mölndal, Sweden*
*[2] Cognitive Disorder Research Unit, Department of Clinical Sciences Malmö, Faculty of Medicine, Lund University, Lund, Sweden*
*[3] Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Faculty of Medicine, Lund University, Lund, Sweden*
*[4] Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*

Alzheimer's disease (AD) is characterised by two major brain changes: plaques of the amyloid beta (Aβ) protein, and tau protein accumulations, i.e. tangles. However, tau protein can form aggregates also independently of Aβ in neurodegenerative diseases called primary tauopathies.

Tau profiles in soluble and insoluble fractions from postmortem brain samples of superior temporal gyrus (STG) and fusiform gyrus (FuG) from patients with primary age-related tauopathy (PART), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), intermediate and late-stage AD were compared to gain knowledge of tau pathological forms in these regions.

Tau from intermediate AD (int-AD, Braak III-IV, n=20), high-AD (Braak V-VI, n=20), CBD/PSP (n=10), and PART (n=12) cases was analysed. Soluble (tris-buffered saline, TBS) and sarkosyl-insoluble (SI) brain extracts were immunoprecipitated with the HT7 antibody. Tryptic peptides were monitored by liquid chromatography/high-resolution data-dependent mass spectrometry using isotope-labelled standards for quantification.

In the SI fraction, tau peptides' levels were much increased in the high-AD group compared to PART in both analysed regions, and the difference was largest for the MTBR peptides. However, in the int-AD group, levels of the same peptides were increased in FuG but not in STG, where they resembled those of the PART and PSP/CBD groups. The phospho-peptides' levels followed the MTBR peptide pattern. In the TBS fraction, the between-group differences were much less pronounced, apart from the phospho-peptides, which were much increased in the high-AD group.

The brain regional difference in abundance levels of the MTBR and phospho-tau peptides is clear in int-AD, but much less pronounced in high-AD, CBD/PSP, and PART, suggesting that FuG is affected earlier in the disease course. Low MTBR peptide accumulation in PART, despite the same Braak stage as int-AD, suggests that tau pathology in PART is much less pronounced.

FINANCIAL SUPPORT: This study was supported by the Cure Alzheimer's Fund, Åhlén-stiftelsen, Stiftelsen för Gamla Tjänarinnor, and Stohnes Stiftelse.

## S26.1. NETWORK-LEVEL REORGANIZATION OF BRAIN FUNCTIONAL CONNECTIVITY AFTER TOTAL AND CHRONIC SLEEP DEPRIVATION

Patrycja Ściślewska[1], Arturo Cabrera Vazquez[2,3,4], Iwona Szatkowska[5], Halszka Kontrymowicz-Ogińska[6], Sophie Achard[2], Aleksandra Domagalik-Pittner[7]

*[1] Faculty of Biology, University of Warsaw, Warsaw, Poland*
*[2] Laboratoire Jean Kuntzmann (LJK), Université Grenoble Alpes, CNRS, Inria, Grenoble INP, Grenoble, France*
*[3] Grenoble Institute of Neuroscience, Inserm U1216, Université Grenoble Alpes, Grenoble, France*
*[4] Toulouse Neuroimaging Center, INSERM U1214, CHU Purpan, Toulouse, France*
*[5] Laboratory of Emotions Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*
*[6] Department of Cognitive Neuroscience and Neuroergonomics, Institute of Applied Psychology, Jagiellonian University, Kraków, Poland*
*[7] Centre for Brain Research, Jagiellonian University, Kraków, Poland*

Although sleep loss is known to impair cognitive performance and emotional regulation, the neural consequences of different types of sleep deprivation remain poorly understood.

In this study, we directly compared acute total sleep deprivation (TSD) and chronic sleep restriction (CSR) to examine how each form of sleep loss alters intrinsic functional brain organization.

In a longitudinal resting-state fMRI design, twenty-eight healthy adults were scanned under three conditions: rested wakefulness (RW), after one night of TSD (~26 h awake), and after five nights of CSR (5 h sleep per night). To quantify disruptions in functional connectivity, we introduced and applied a within-subject Hub Disruption Index, a novel graph-based measure of individual neural reorganization. We further characterized condition-specific effects using Covariate-Constrained Manifold Learning (CCML). Finally, to compare neural changes with subjective experiences, we used psychometric assessment of sleep quality, sleepiness and circadian traits.

Both TSD and CSR led to consistent reorganization of network topology relative to RW but affected distinct functional hubs. Regional changes were most prominent within subsystems of the default mode, frontoparietal, and cerebellar networks. CCML embeddings further supported divergent connectivity patterns between TSD and CSR. Additionally, greater subjective sleepiness was associated with reduced network integration during RW, and circadian phenotype emerged as a key determinant of individual sensitivity to sleep loss.

Acute and chronic sleep loss induce distinct alterations in human functional brain organization. These findings advance mechanistic understanding of sleep deprivation and offer new insights for clinical interventions.

FINANCIAL SUPPORT: Polish National Science Centre No.: 2018/29/B/HS6/01934, France 2030 program, ANR-23-IACL-0006.

## S26.2. NEUROANATOMY OF LUCID DREAMING FREQUENCY: A LARGE-SCALE QUANTITATIVE MRI STUDY

Karolina Raczek[1,2,3], Renate Rutiku[4], Patrycja Ściślewska[5], Aleksandra Domagalik-Pittner[2], Michał Wierzchoń[2], Martin Dresler[3]

*[1] Doctoral School in the Social Sciences, Jagiellonian University, Kraków, Poland*

*[2] Centre for Brain Research, Jagiellonian University, Kraków, Poland*

*[3] Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Center, Nijmegen, The Netherlands*

*[4] Consciousness Lab, Institute of Psychology, Jagiellonian University, Kraków, Poland*

*[5] Institute of Experimental Zoology, Faculty of Biology, University of Warsaw, Warsaw, Poland*

Becoming aware of the current dream state during ongoing sleep is referred to as lucid dreaming (LD), a phenomenon that shows promise for both clinical and scientific applications. Although LD is infrequent for most, its frequency varies substantially across individuals. While progress has been made, a comprehensive understanding of the neural underpinnings associated with LD remains elusive, with conflicting evidence regarding whether LD is linked to neuroanatomical differences.

As part of the NeuralArchCon COST Action, we characterized a large sample of participants (N=288). LD frequency was assessed using the Dream Recall Questionnaire, while the brain's microstructure was quantified via multiparametric mapping of magnetization transfer (MT), proton density (PD), longitudinal relaxation rate (R1), and transverse relaxation rate (R2*). We performed whole-brain volumetric analyses of these quantitative maps separately for grey and white matter using SPM12 and the hMRI toolbox. Additionally, region-of-interest analysis was conducted on the grey matter R1 maps using 5-mm spherical ROIs generated with the MarsBaR toolbox in SPM12, focusing on the anterior prefrontal cortex, anterior cingulate cortex, supplementary motor area, and hippocampus. The statistical model controlled for age, sex, total intracranial volume, and general dream recall frequency.

No significant associations between LD frequency and brain structure were observed (all cluster-level $p > 0.05$, FDR corrected). Furthermore, a targeted ROI analysis also yielded no significant associations.

As the largest investigation to date, this study provides new evidence that LD frequency is not associated with specific structural brain differences, suggesting that the phenomenon may instead rely on functional neural dynamics.

## S26.3. SLEEP STAGES AFFECT LOW-GAMMA RANGE EFFECTIVE CORTICAL CONNECTIVITY FOR 40-HZ AUDITORY STEADY-STATE RESPONSES

Anna Zofia Leśniewska[1,2], Urszula Górska-Klimowska[1,3], Mirosław Wyczesany[1], Małgorzata Hołda[1], Bartłomiej Chojnacki[4], Marek Binder[1]

[1] *Institute of Psychology, Jagiellonian University, Kraków, Poland*

[2] *Faculty of Psychological and Educational Sciences, Université Libre de Bruxelles, Brussels, Belgium*

[3] *Department of Psychiatry, University of Wisconsin–Madison, Madison, United States*

[4] *Department of Mechanics and Vibroacoustics, Faculty of Mechanical Engineering and Robotics, AGH University of Kraków, Kraków, Poland*

The 40-Hz auditory steady-state response (ASSR) is a sensitive marker of arousal fluctuations, which has been reported to decrease during deep sleep. However, sleep-related changes in directional connectivity during 40-Hz ASSR remain underexplored.

In this study, we examined how sleep stages affect the 40-Hz ASSR propagation. We focused on the connections between auditory and associative regions in the temporal, prefrontal, and temporo-parietal cortices. We hypothesized that: 1) feedback connections from associative to primary auditory areas will be the most affected by the arousal state changes; 2) associative reciprocal connectivity between prefrontal and temporo-parietal regions will display gradual connectivity reduction with increasing NREM sleep depth, with partial restoration during REM sleep.

EEG data during periodic 40-Hz auditory stimulation were collected during an overnight study from 29 normal-hearing human subjects (including 16 females). Source analysis was used to locate cortical activity, and effective connectivity was assessed using the Directed Transfer Function (DTF) in the 37–43 Hz frequency range.

Our results demonstrated that during deeper sleep (NREM N2 and N3, and REM), both feedforward and feedback connectivity were significantly reduced to a comparable extent. At sleep onset (NREM N1), feedforward disruptions were already robust despite affecting a limited number of pathways, whereas feedback reductions were weaker and increased progressively across REM, NREM N2, and N3 sleep, contradicting our first hypothesis. Our second hypothesis was confirmed: reciprocal connectivity between the prefrontal and parietal associative regions was significantly weakened with increasing sleep depth.

Overall, the reduced propagation of neural signals underlying the 40-Hz ASSR indicates that a marked and widespread breakdown of intracortical effective connectivity emerges after sleep onset and becomes fully pronounced in deeper sleep stages.

FINANCIAL SUPPORT: This study was supported by the Polish National Science Centre under awards number 2018/31/B/HS6/03920 (MB, UG-K, AL) and 2018/31/G/HS6/02490 (MW, AL).

## S26.4. PUPILLARY LIGHT REFLEX RECOVERY TIME AS A CANDIDATE MARKER OF EMOTION DYSREGULATION IN SLEEP-ONSET INSOMNIA

Marcin Iwański

*Third Department of Psychiatry, Affective Disorders Unit and Sleep Medicine Centre, Institute of Psychiatry and Neurology, Warsaw, Poland*

Emotion dysregulation is increasingly recognized as a contributor to sleep-onset insomnia. Its relationship with objective physiological markers remains unexplored. We investigated the association between pupillary light reflex (PLR) parameters and self-reported emotion dysregulation in this population.

Forty-six patients (32 female; mean age 50.0 ± 14.3 years) with sleep-onset insomnia were assessed at baseline of group CBT-I. Patients completed the DERS-18, PHQ-9, GAD-7, ISI, and DBAS-16. Pupillometry (Neuroptics PLR-3000) was attempted in all patients following 10-minute dark adaptation using a single-trial protocol; valid data were available for 31 patients. Eight PLR parameters were extracted, including the 75% recovery time (T75).

T75 correlated positively with DERS-18 ($r=+.28$, 95% CI [−.19, +.64]; Spearman $\rho=+.29$). The bootstrap 95% CI (5000 iterations) was [−.07, +.61]. In a regression model including age, baseline diameter (INIT), and percent constriction (DELTA) as covariates, T75 showed the largest standardised coefficient ($\beta=+.36$; $\Delta R^2=.11$; Cohen's $f^2=.15$). Among the eight PLR parameters examined, T75 was the only parameter with $|r| > .20$ with DERS-18; the remaining seven showed $|r| \leq .19$. A median split on DERS-18 yielded Cohen's $d=0.44$. All confidence intervals for the T75–DERS-18 association included zero.

The selective association of DERS-18 with T75 observed in this pilot analysis — but not with afferent, constriction-phase, or sympathetic-dominated PLR pa-

rameters — is consistent with the theoretical prediction that emotion regulation maps onto the recovery phase of the PLR, and warrants formal testing. Adequately powered pre-registered replication (n ≈ 85 for 80% power at r=.30) is warranted to formally test the autonomic-flexibility hypothesis in insomnia.

## S26.5. OPPOSING DIURNAL TRAJECTORIES OF DOPAMINE D2 AND MU-OPIOID RECEPTOR AVAILABILITY AND THEIR SEASONAL MODULATION IN THE HUMAN BRAIN

Michal Rafal Zareba[1], Juha Rinne[2,3], Jarmo Hietala[2,3,4], Pirjo Nuutila[2,3,5], Lauri Nummenmaa[2,3,6], Lihua Sun[2,3,7]

[1] *Department of Basic and Clinical Psychology and Psychobiology, Jaume I University, Castellón de la Plana, Spain*

[2] *Turku PET Centre, University of Turku, Turku, Finland*

[3] *Turku PET Centre, Turku University Hospital, Turku, Finland*

[4] *Department of Psychiatry, Turku University Hospital, Turku, Finland*

[5] *Department of Endocrinology, Turku University Hospital, Turku, Finland*

[6] *Department of Psychology, University of Turku, Turku, Finland*

[7] *Turku Collegium for Science, Medicine and Technology, University of Turku, Turku, Finland*

Diurnal variability in arousal, motivation and cognitive performance are known to be modulated by seasonal differences in light exposure, with the intricate connections between these phenomena being especially evident in seasonal affective disorder.

Considering the role of the dopaminergic and opioidergic systems in the aforementioned cognitive processes, the current work used a large positron emission tomography (PET) dataset (n=377) to explore the presence of time-of-day (TOD)-dependent fluctuations in receptor availability, considering the previously reported effects of seasonality and subclinical anxiety.

The data revealed decreased dopamine type-2 (D2R) and increased mu-opioid receptor (MOR) availability in the afternoon compared to morning. The impact of the interactions with the seasonal daylength changes was, however, observed only for MOR, mapping onto brain networks implicated in emotion processing, affective regulation and social cognition. Interestingly, the MOR availability in the aforementioned regions was also sensitive to the interactions of TOD and individual anxiety levels.

As such, the current results reveal the diurnal variability in indices of dopaminergic and opioidergic neurotransmission by highlighting the opposing trajectory between the two systems. Furthermore, the data pinpoints the special role that the opioid system may play in linking together diurnal and seasonal rhythms with affective and anxiety disorders.

FINANCIAL SUPPORT: Michal Rafal Zareba received financial support from the Fundación Balaguer-Gonel Hermanos in the form of a mobility grant. Lauri Nummenmaa was supported by the European Research Council Advanced Grant (101141656).

# POSTERS

## S1P01. DUAL-COLOR FIBER PHOTOMETRY REVEALS S1-M1 POPULATION DYNAMICS DURING JOYSTICK-BASED SENSORIMOTOR ADAPTATION IN HEAD-FIXED MICE

Martyna Gorkowska-Nosal[1,2], Natalia Kurek[1], Tomasz Błasiak[1], Przemysław E. Cieślak[1]

*[1] Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

*[2] Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Sensorimotor adaptation updates ongoing movements based on error feedback, but the population dynamics linking primary somatosensory cortex (S1) input to primary motor cortex (M1) during adaptation remain poorly defined.

AIM(S): To track kinematics and cortical activity across learning and sensorimotor adaptation stages, we used a mouse joystick task with controlled force perturbations.

METHOD(S): Head-fixed mice were trained to pull a joystick for reward. A lateral force perturbation was introduced using a magnetic force field. We performed dual-color fiber photometry through an optical fiber implanted in M1 to simultaneously record calcium activity in S1-innervated M1 neurons (jRGECO) and S1 axonal input in M1 (GCaMP). Signals were analyzed during skill acquisition, stable task performance, perturbation onset, adaptation, and washout after perturbation removal, together with joystick kinematic parameters.

RESULTS: Preliminary data indicate that mice show progressive behavioural compensation after perturbation onset and express a washout aftereffect after perturbation removal, consistent with sensorimotor adaptation. Kinematic changes across task phases were accompanied by movement-related calcium signals in both recorded channels. Initial analyses suggest that the temporal profiles of S1-related input signals and M1 population activity are not identical and may change across adaptation stages.

CONCLUSIONS: These results support the feasibility of combining a joystick-based force-field adaptation task with dual-color fiber photometry to study cortical circuit dynamics in mice. Ongoing analyses will determine how changes in S1-M1 activity relate to specific kinematic features and adaptation dynamics.

FINANCIAL SUPPORT: National Science Centre, Poland – Preludium 23, 2024/53/N/NZ4/04146.

## S1P02. NR2B-DEPENDENT SYNAPTIC PLASTICITY IN THE CENTRAL AMYGDALA DURING APPETITIVE LEARNING

Justyna Wisniewska, Anna Beroun

*Laboratory of Neuronal Plasticity, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: The brain is a highly dynamic structure capable of adapting to environmental changes and encoding experiences as memories. Learning involves activity-dependent modifications of synaptic connectivity, including the formation of new synapses through long-term potentiation (LTP) and the elimination of existing ones via long-term depression (LTD). Both processes can transiently produce glutamatergic synapses that are functionally silent, characterized by the presence of NMDA receptors and the absence of AMPA receptors. Our previous work demonstrated the formation of silent synapses in the central nucleus of the amygdala (CeA) during appetitive learning in mice. However, the underlying mechanisms—specifically whether these synapses result from LTP or LTD—remain unclear.

AIM(S): This study aimed to evaluate the role of LTP in appetitive learning.

METHOD(S): by selectively knocking out the NR2B subunit of the NMDA receptor in the CeA, thereby impairing LTP. Behavioral assay and electrophysiological patch-clamp recordings were performed on NR2B knockout mice.

RESULTS: Behavioral assays in the IntelliCage system revealed a decreased sucrose preference in NR2B knockout mice compared with wild-type controls, indicating altered appetitive behavior. Complementary electrophysiological recordings demonstrated significant changes in NMDA receptor–mediated current kinetics, consistent with modified synaptic function in the CeA.

CONCLUSIONS: Our results indicate a significant alteration in neuronal functional activity.

FINANCIAL SUPPORT: Narodowe Centrum Nauki 2022/47/B/NZ4/02787.

## S1P03. DIVERGENT SEEKING AND CONSUMPTION PATTERNS FOR SUCROSE, QUININE, AND METHAMPHETAMINE IN AN AUTOMATED INTELLICAGE SELF-ADMINISTRATION PARADIGM

Nupur Ravindra Mahadeokar, Anna Beroun

*Laboratory of Neuronal Plasticity, BRAINCITY, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Decision-making between rewards is shaped by both reward value and associated sensory cues. Understanding how seeking and consumption are balanced can shed light on mechanisms underlying motivation, reward preference, and addiction-related behaviors.

AIM(S): To investigate how cue-associated rewards (sucrose, quinine, methamphetamine) influence motivational (nosepoke) versus consummatory (licking) behavior and to examine preference shifts and relapse patterns in a group-housed, automated behavioral paradigm.

METHOD(S): Animals were housed in IntelliCage, which continuously recorded corner visits, nosepokes, and licking. After habituation and baseline preference assessment, reward phases included sucrose, quinine, and meth, each paired with LED cue in defined time windows and separated by water-only washout periods. Preference, nosepoke-to-lick ratios, and relapse behaviors were tracked throughout.

RESULTS: Sucrose access produced low nosepoke-to-lick ratios, reflecting efficient action–consumption coupling. Meth and quinine elicited high ratios, indicating increased seeking but reduced consumption. Corner preference patterns also suggested hierarchy-related access differences.

CONCLUSIONS: Reward type and cue association strongly modulate the balance between seeking and consumption. Natural rewards favor consumption-driven responding, whereas drug and aversive stimuli bias toward seeking. The automated IntelliCage paradigm effectively dissociates motivational and consummatory behavior and provides a platform for studying reward choice, behavioral flexibility, and addiction-related neural plasticity.

FINANCIAL SUPPORT: National Science Center, Poland. Grant Number: Sonata Bis 2023/50/E/NZ4/00421.

## S1P04. ASSESSMENT OF LEADER-FOLLOWER DYNAMICS IN POSTCOPULATORY TANDEMS OF THE COMMON FIREBUG PYRRHOCORIS APTERUS

Jagoda Nawrocka

*Inter-faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warsaw, Poland*

INTRODUCTION: Firebugs (Pyrrhocoris apterus), common hemipteran insects, remain physically connected in post-copulatory tandems for periods ranging from two hours to seven days, a behavior thought to function as mate guarding. During this time, pairs actively navigate their environment and respond to external stimuli, including rapidly disengaging when threatened.

AIM(S): The dynamics and functional consequences of this behavior are largely understudied. Tandem locomotion in firebugs provides a natural system for studying distributed sensorimotor control and coordination between physically coupled individuals. The aim of this study was to quantify the kinematics of tandem movement and determine whether locomotor leadership is biased toward one sex and/or influenced by body size.

METHOD(S): Connected pairs collected in the wild (N=41) and raised in controlled conditions (N=10) were recorded for 15 minutes in a circular arena. Video recordings were analyzed using a DeepLabCut-based pose estimation model. Locomotor parameters including speed, traveled distance, trajectory, and angles between body parts were extracted and analyzed.

RESULTS: Preliminary analyses indicate strong female-biased leadership during tandem locomotion, with females contributing most of the traveled distance and directional changes. The influence of body size remains inconclusive, as females are generally larger than males, limiting independent assessment of size effects.

CONCLUSIONS: These findings suggest that tandem locomotion in firebugs is characterized by asymmetric movement control, potentially reflecting evolutionary trade-offs and biomechanical limitations. Ongoing analyses of leg kinematics, antennal movement, and behavioral unsupervised clustering will further clarify coordination strategies and behavioral states during prolonged post-copulatory association.

FINANCIAL SUPPORT: Self-funded.

## S1P05. EFFECTS OF LONG-TERM USE OF 3-HYDROXY-3-METHYLGLUTARYL–COENZYME A REDUCTASE INHIBITORS (STATINS) ON COGNITION AND GLUCOSE METABOLISM IN HYPERCHOLESTEROLEMIC MOUSE MODEL

Emilia Czabrowska[1], Joanna Osika[1], Oliwia Kmieć[1], Hanna Smukowska[1], Iga Łobińska[1], Aniela Rękawek[1], Przemysław Kowiański[1,2], Grażyna Lietzau[1]

[1] *Division of Anatomy and Neurobiology, Faculty of Medicine, Medical University of Gdańsk, Gdańsk, Poland*
[2] *Institute of Health Sciences, Pomeranian University in Słupsk, Słupsk, Poland*

INTRODUCTION: Statins are widely prescribed for dyslipidemia. Some evidence links long-term statin use to an increased risk of type 2 diabetes mellitus (T2DM) and cognitive impairment.

AIM(S): This study aimed to assess whether long-term statin use induces or exacerbates T2DM and affects memory and learning processes in hypercholesterolemic and non-hypercholesterolemic mice.

METHOD(S): Four-month-old male C57BL/6J mice were randomly assigned to one of two groups: SD (standard diet-fed, n=20) and HCD (high-cholesterol diet-fed, n=28). After 5 months, the mice were gavaged with either simvastatin (SMV, 5 mg/kg) or vehicle (VEH, 0.9% NaCl) for 20 weeks and assigned to four groups: SDVEH, SDSMV, HCDVEH, HCDSMV. To assess if SMV affects diabetic-related parameters, fasting glucose and insulin, insulin tolerance test, and HOMA index were evaluated. Cognitive functions were assessed after 12 weeks using Barnes Maze (BM) and Novel Object Recognition Test (NORT).

RESULTS: Results showed an elevated HOMA-IR value (>1) in SDSMV and SDVEH mice, suggesting mild insulin resistance. This effect was age-related. Glucose levels were elevated in both HCD groups, with no difference between them, indicating that SMV did not affect hyperglycemia. However, HCDSMV mice had significantly lower HOMA-IR compared with HCDVEH (2.7±0.62 *vs.* 4.2±1.89, p=0.02), indicating that SMV improves insulin sensitivity. BM results showed that HCDSMV mice, compared to HCDVEH mice, had decreased escape latency over the three-day period (p=0.002, p=0.0006 and p=0.01), which suggests that SMV improves spatial learning. Additionally, HCDVEH mice demonstrated impairment of short-term reference memory (p=0.006). SMV administration did not alter this parameter. No group differences were observed in the NORT.

CONCLUSIONS: In conclusion, long-term simvastatin administration did not induce or exacerbate T2DM but improved insulin sensitivity. Additionally, simvastatin reversed HCD-induced spatial learning deficits but had no effect on reference and recognition memory.

FINANCIAL SUPPORT: This study was supported by the National Science Centre (NCN): 2023/07/X/NZ7/01480.

## S1P06. THE ROLE OF THE MEDIAL PREFRONTAL CORTEX IN A SHORT- AND LONG-TERM SPATIAL MEMORY TASK IN RATS

Chiara Roth, Jozsef Csicsvari

*Institute of Science and Technology Austria (ISTA), Klosterneuburg, Austria*

INTRODUCTION: The medial prefrontal cortex (mPFC) is involved in numerous cognitive functions, including memory processing. While substantial evidence links the mPFC to short-term memory maintenance, its role in long-term memory processes remains less understood. Spatial memories are thought to initially be encoded in the entorhinal-hippocampal circuit, and consecutively be transferred into the cortex via memory consolidation. In the mPFC space is represented in an abstract form, as opposed to the spatial representation by so-called place cells in the hippocampus. A substantial proportion of mPFC cells exhibits activity related to rewards and reward expectancy.

AIM(S): We investigate how the rat mPFC contributes to spatial memory across different retention intervals. We examine at what stage place-related activity emerges in relation to the spatial learning, and test whether reward locations are disproportionally represented in the mPFC at different times.

METHOD(S): For this, we perform extracellular recordings in the prelimbic area of the mPFC in freely moving rats with chronically implanted Neuropixels probes. Electrophysiological and behavioral data is collected before and throughout the entire learning phase, and during short- and long-term memory retrieval phases of an adapted cheeseboard task.

## S1P07. THE ROLE OF SLEEP IN REGULATING OXIDATIVE STRESS LEVELS IN THE BRAIN

Pola Szerszon, Milena Damulewicz

*Institute of Zoology and Biomedical Research, Department of Cell Biology and Imaging, Kraków, Poland*

INTRODUCTION: Sleep is essential in neuronal homeostasis and overall well-being of the organisms. One of the processes influenced by sleep is mitochondrial metabolism, which is a major source of reactive oxygen species (ROS) in neurons. Free radicals are created in mitochondria during cellular respiration in the electron transport chain. Mitochondria are highly dynamic organelles whose functional state can be monitored using the genetically encoded reporter mitoTimer. Consequently, with the rising level of oxidative stress in the mitochondria, color gradually shifts from green to red fluorescence over time.

AIM(S): The research aimed to investigate whether acute sleep deprivation affects oxidative stress levels in the sleep and memory center, called mushroom bodies of Drosophila melanogaster.

METHOD(S): Using elav-GAL4 driver, mitoTimer was expressed pan-neuronally in Drosophila melanogaster. 4-day-old male flies were selected and subjected to mechanical sleep deprivation during the night. Control flies were maintained under standard conditions. Following brain dissection, confocal imaging of mushroom bodies (MB) was performed under identical acquisition settings. Both green and red fluorescence were quantified using ImageJ software, and oxidative stress was expressed as the red/green ratio for each brain.

RESULTS: Sleep deprivation increases the red/green fluorescence ratio compared to the control group.

CONCLUSIONS: The results suggest that acute sleep loss disrupts neuronal homeostasis in MB, which are critical areas responsible for learning and memory.

## S1P08. CAMK2B AND SMAP1 AS NOVEL BRAIN PROTEIN BIOMARKERS LINKED TO LONG-TERM ABSTINENCE FOLLOWING EXTENDED COCAINE SELF-ADMINISTRATION

Joanna Jastrzębska[1], Saiyara Aniqa[1], Kinga Szafran-Pilch[2], Anna Drabik[3], Renata Pieniążek[1], Joanna Wierońska[4], Piotr Brański[4], Grzegorz Burnat[4], Małgorzata Frankowska[1], Przemek Mielczarek[2,3], Małgorzata Filip[1]

[1] *Department of Drug Addiction Pharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Laboratory of Proteomics and Mass Spectrometry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[3] *Faculty of Materials Science and Ceramics, AGH University of Kraków, Kraków, Poland*

[4] *Department of Neurobiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Cocaine addiction is a chronic brain disorder and remains a major public health problem, with craving and relapse as core clinical features.

AIM(S): This study aimed to identify brain proteins regulated after prolonged abstinence following extended cocaine self-administration in rats using advanced proteomic approaches.

METHOD(S): Male and female Wistar Han rats were surgically implanted with jugular vein catheters and trained to self-administer cocaine under fixed-ratio schedules (FR1–FR5) with light and sound cues. Animals underwent short (1 h) followed by extended (6 h) access sessions. Yoked cocaine and saline controls were used to dissociate motivational from pharmacological effects. After 30 days of withdrawal in the home cage, the dorsal striatum and prefrontal cortex were collected and analyzed using SP3-based sample preparation and DIA mass spectrometry. Differentially regulated proteins were used to design structure-specific aptamers. A separate group of rats was injected intravenously with Cy5-labeled aptamers, and whole-body fluorescence was quantified by in vivo imaging using the IVIS Lumina III system (Revvity) at 1, 2, and 24 h to assess biodistribution and clearance.

RESULTS: Proteomic profiling revealed robust, region-specific protein regulation in relapse-related circuits after prolonged abstinence in both sexes. Sex-dependent targets were identified, with CAMK2B predominant in males and SMAP1 in females. In vivo imaging showed rapid systemic distribution of both aptamers within 1 h, with distinct regional accumulation patterns (CAMK2B, enhanced cranial signal; SMAP1, stronger abdominal signal), followed by partial tissue retention at 2 h and marked signal reduction by 24 h, consistent with progressive clearance.

CONCLUSIONS: The study identifies novel, partially sex-specific protein biomarkers associated with prolonged abstinence after cocaine self-administration and demonstrates the feasibility of aptamer-based in vivo target engagement and biodistribution profiling.

FINANCIAL SUPPORT: This research was funded by the Medical Research Agency (ABM) grant number 2024/ABM/03/KPO/KPOD.07.07-IW.07-0104/24.

## S1P09. FUNCTIONAL INTERACTION OF 5-HT7 AND NMDA RECEPTORS IN STRESS-INDUCED BEHAVIORAL AND SYNAPTIC ALTERATIONS

Zeinab Bedrood[1], Monika Bijata[1], Izabela Figiel[1], Kim Dore[2], Bernadeta Szewczyk[3], Jakub Wlodarczyk[1]

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

*[2] Center for Neural Circuits and Behavior, Department of Neuroscience and Section for Neurobiology, Division of Biology, University of California at San Diego, San Diego, United States*

*[3] Department of Neurobiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Major depressive disorder involves impaired synaptic plasticity and dysregulated serotonergic and glutamatergic signaling. Interactions between serotonin 5-HT7 receptors (5-HT7R) and N-methyl-D-aspartate receptors (NMDAR) may underlie stress-induced behavioral alterations; however, their direct interaction and functional relevance in stress-related pathology remain unclear.

AIM(S): To investigate physical and functional interactions between 5-HT7R and NMDAR and evaluate behavioral effects of pharmacological modulation in a treatment-resistant depression model.

METHOD(S): Receptor interactions were assessed in hippocampal neurons using co-localization analysis and fluorescence recovery after photobleaching (FRAP). Mice exposed to chronic immobilization stress combined with a zinc-deficient diet were treated with the NMDAR antagonist Nitrosynapsin or the multimodal antidepressant vortioxetine (VTX). Behavioral performance was evaluated using the tail suspension test (TST), novelty-suppressed feeding test (NSFT), and object location test (OLT).

RESULTS: Co-localization revealed substantial overlap between 5-HT7R and NMDAR. FRAP showed altered NMDA receptor mobility in neurons co-expressing 5-HT7R, indicating functional interaction. Nitrosynapsin reduced immobility and anxiety-like behavior to control levels. VTX decreased latency to feed in the NSFT and improved OLT performance, reversing stress-induced deficits.

CONCLUSIONS: These findings demonstrate spatial proximity and altered mobility of 5-HT7R and NMDAR in dendrites. Convergent behavioral effects of Nitrosynapsin and VTX suggest overlapping regulatory mechanisms underlying stress-induced neuronal dysfunction. Together, the receptors may act as a unified signaling module contributing to dendritic signaling associated with stress-related behavioral alterations.

FINANCIAL SUPPORT: This research was funded by the National Science Centre grant number 2021/41/B/NZ4/02603.

## S1P10. DIFFERENTIAL EFFECTS OF COBALT CHLORIDE, INTERMITTENT HYPOXIA, AND BAROMETRIC CHAMBER ON PTSD-LIKE BEHAVIOUR IN RATS

Denys Porkhalo[1], Mariia Kozlovska[2], Victor Dosenko[1]

*[1] Department of General and Molecular Pathophysiology, Bogomoletz Institute of Physiology of NAS of Ukraine, Kyiv, Ukraine*

*[2] Department of Hypoxic States, Bogomoletz Institute of Physiology of NAS of Ukraine, Kyiv, Ukraine*

INTRODUCTION: Post-Traumatic stress disorder (PTSD) is a psychiatric issue caused by traumatic events like war, violence, or natural disasters. Its symptoms and brain mechanisms are well-understood, but the molecular pathways involved in its development and recovery remain unknown. We hypothesise that hypoxia and hypoxia-induced factors may play a role in reducing PTSD symptoms by influencing BDNF, as studies show intermittent hypoxia increases BDNF expression.

AIM(S): Our goal was to investigate how different types of hypoxia can affect PTSD-like behaviour in rats. In this way, we wanted to understand whether there is a need to study in more detail the molecular mechanisms of hypoxia's effect on the formation of PTSD and the restoration of physiological behaviour.

METHOD(S): The first group received oral cobalt chloride (15 mg/kg) once daily for the week. The second was in a hypoxic chamber with 14% of oxygen each day for a week. Hypobaric hypoxia was reproduced by 'lifting' animals in a barometric chamber to an 'altitude' up to 4500 meters. Each group had 24 rats, split into control and PTSD-like groups. To model PTSD behaviour in rats, we have used single-prolonged stress methods. Behavioural tests included the Open Field, Dark-Light Box Test, and Elevated Plus Maze.

RESULTS: PTSD animals showed elevated anxiety, which was reduced by intermittent hypoxia and hypobaric chamber exposure: light zone occupancy recovered by 57% and 90% respectively ($p<0.05$), corner time normalized to 3.9-fold ($p<0.05$), and erratic locomotion decreased with path efficiency improving by 93% ($p<0.05$), indicating that hypoxic interventions effectively attenuate core PTSD-like behavioural symptoms.

CONCLUSIONS: Throughout the three methods, intermittent hypoxia consistently influenced PTSD-like rats by boosting exploration and reducing avoidance. In control rats, it had opposite effects, suggesting a modulatory role in behaviour. This highlights the need for further research into the molecular pathways of hypoxia's impact on PTSD mechanisms.

FINANCIAL SUPPORT: One of co-authors was supported by IC AMER of NAS of Ukraine.

## S1P10. DIFFERENTIAL EFFECTS OF COBALT CHLORIDE, INTERMITTENT HYPOXIA, AND HYPOBARIC HYPOXIA ON PTSD-LIKE BEHAVIOUR IN RATS

Denys Porkhalo[1], Mariia Kozlovska[2], Victor Dosenko[1]

*[1] Department of General and Molecular Pathophysiology, Bogomoletz Institute of Physiology of NAS of Ukraine, Kyiv, Ukraine*

*[2] Department of Hypoxic States, Bogomoletz Institute of Physiology of NAS of Ukraine, Kyiv, Ukraine*

INTRODUCTION: Post-Traumatic stress disorder (PTSD) is a psychiatric issue caused by traumatic events like war, violence, and natural disasters. Its symptoms and brain mechanisms are well-understood, but the molecular pathways involved in its development and recovery remain unknown. We hypothesise that hypoxia may play a role in reducing PTSD symptoms by influencing BDNF, as studies show intermittent hypoxia increases BDNF expression.

AIM(S): Our goal was to investigate how different types of hypoxia affect PTSD-like behaviour in rats, to see if studying the molecular mechanisms of hypoxia's impact on PTSD development and physiological recovery is necessary.

METHOD(S): The first group received oral cobalt chloride (15 mg/kg) once daily for the week. The second was in a hypoxic chamber with 14% of oxygen each day for a week. Hypobaric hypoxia was reproduced by 'lifting' animals in a barometric chamber to an 'altitude' up to 4500 meters. Each group had 24 rats, split into control and PTSD-like groups. To model PTSD behaviour in rats, we have used single-prolonged stress methods. Behavioural tests included the Open Field, Dark-Light Box Test, and Elevated Plus Maze.

RESULTS: PTSD animals showed elevated anxiety, which was reduced by intermittent hypoxia and hypobaric chamber exposure: light zone occupancy recovered by 57% and 90% respectively ($p<0.05$), corner time normalized to 3.9-fold ($p<0.05$), and erratic locomotion decreased with path efficiency improving by 93% ($p<0.05$), indicating that hypoxic interventions effectively attenuate core PTSD-like behavioural symptoms.

CONCLUSIONS: Throughout the three methods, intermittent hypoxia consistently influenced PTSD-like rats by boosting exploration and reducing avoidance. In control rats, it had opposite effects, suggesting a modulatory role in behaviour. This highlights the need for further research into the molecular pathways of hypoxia's impact on PTSD mechanisms.

FINANCIAL SUPPORT: One of the co-authors was funded by IC AMER of NAS of Ukraine.

## S1P11. PZM21, A G-PROTEIN BIASED M-OPIOID AGONIST, REDUCES OXYCODONE SEEKING IN PROTRACTED BUT NOT EARLY WITHDRAWAL IN RATS

Anna Ciejka[1], Barbara Jędrzejewska[2], Daria Krzysztofik[1], Justyna Furso[2], Ryszard Bugno[3], Ryszard Przewłocki[1], Wojciech Solecki[2]

*[1] Department of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Science*

*[2] The Department of Neurobiology and Neuropsychology, Institute of Applied Psychology, Faculty of Management and Social Communication, Jagiellonian University, Cracow, Poland*

*[3] Department of Medicinal Chemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Opioid use disorder poses a pressing clinical problem, as currently available substitution therapies retain abuse liability. The G protein-biased μ-opioid receptor agonist PZM21 has been proposed as a safer alternative due to its reduced reinforcing properties. However, its effects on opioid-seeking behavior across different stages of withdrawal and relapse remain unexplored.

AIM(S): This study examined whether PZM21 modulates oxycodone-seeking during early (withdrawal day 1; WD1) and protracted (WD30) abstinence, and reinstatement, in rats.

METHOD(S): Male Sprague–Dawley rats were trained to self-administer intravenous oxycodone under a fixed ratio schedule of reinforcement, in which active lever presses resulted in drug infusion. Following self-administration training, animals underwent abstinence,

and after PZM21 (10-40 mg/kg) intraperitoneal administration, they were tested for oxycodone-seeking during WD1 or WD30. After extinction training, reinstatement testing allowed active/inactive lever access (no drug infusion) and elicited oxycodone seeking via oxycodone prime, followed by PZM21 (10-40 mg/kg). In control tests, locomotor activity and anxiety-like behavior were assessed using the open field test.

RESULTS: During WD1, PZM21 did not alter overall active/inactive responding, though the time course showed a persistent reduction in active lever responding. In contrast, during WD30, PZM21 reduced active lever responding without affecting inactive lever presses. Both 10 and 40 mg/kg doses reduced oxycodone seeking during reinstatement tests. PZM21 did not affect locomotor activity or anxiety-like behavior.

CONCLUSIONS: PZM21 reduced oxycodone-seeking during protracted withdrawal and reinstatement—but not early withdrawal—without locomotor/anxiety effects, suggesting a stage-dependent effect and a promise for biased agonists in substitution/maintenance therapy.

FINANCIAL SUPPORT: National Science Centre, UMO-2020-39-B-NZ7-03537.

## S1P12. SEX-DEPENDENT EFFECTS OF THE KAPPA-OPIOID RECEPTOR-BIASED AGONIST NALFURAFINE AND G PROTEIN-BIASED M-OPIOID RECEPTOR AGONIST PZM21 ON PHASIC DOPAMINE RELEASE IN RATS

Magdalena Chodlewska[1], Jakub Duran[1], Daria Krzysztofik[2], Jakub Bilnicki[3], Ryszard Bugno[4], Justyna Furso[2], Ryszard Przewłocki[2], Wojciech Solecki[1]

*[1] Department of Neurobiology and Neuropsychology, Institute of Applied Psychology, Faculty of Management and Social Communication, Jagiellonian University, Kraków, Poland*

*[2] Department of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Science, Kraków, Poland*

*[3] Department of Neurobiology and Neuropsychology, Institute of Applied Psychology, Faculty of Management and Social Communication, Jagiellonian University, Kraków, Poland*

*[4] Department of Medicinal Chemistry, Institute of Applied Psychology, Faculty of Management and Social Communication, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Biological sex influences vulnerability to neuropsychiatric disorders, including opioid use disorder and mood disorders. Males and females show distinct behavioral responses to opioids, driven by differential modulation of mesolimbic dopamine signaling. μ-Opioid receptors (MORs), primary mediators of analgesia and reward, exhibit sex-dependent effects. Females show heightened sensitivity to MOR-mediated euphoria and reinforcement, increasing addiction liability, whereas males display stronger dysphoric responses to kappa-opioid receptor (KOR) activation, promoting negative affect and depressive-like states. Nalfurafine, a selective biased KOR agonist, preferentially activates G protein signaling while avoiding β-arrestin pathways, providing analgesia with reduced sedation, dysphoria, and aversive effects. PZM21, a biased MOR agonist, favors G protein signaling over β-arrestin recruitment, attenuating reinforcing properties and lowering addiction liability of conventional MOR agonists.

AIM(S): We investigated whether nalfurafine and PZM21 modulate phasic dopamine release in the mesolimbic system and whether these effects differ by sex.

METHOD(S): Phasic dopamine release was measured using fast-scan cyclic voltammetry in urethane-anesthetized male and female Sprague Dawley rats. Dopamine transients were evoked by electrical stimulation of the ventral tegmental area (VTA) and recorded in the nucleus accumbens (NAc). Nalfurafine and PZM21 were injected into the VTA to assess their impact on dopamine release.

RESULTS: Both nalfurafine and PZM21 attenuated phasic dopamine release in the NAc across sexes. However, the response in females was weaker than in males, indicating a diminished effect of these biased agonists. Suggesting reduced sensitivity to KOR and MOR agonism in the female brain.

CONCLUSIONS: These findings highlight sex dependent modulation of mesolimbic dopamine by biased KOR and MOR agonists, emphasizing sex as an important biological variable in opioid research with direct clinical implications.

FINANCIAL SUPPORT: National Science Centre: UMO-2020/39/B/NZ7/03537.

## S1P13. EFFECT OF TWO NOVEL BIASED M-OPIOID RECEPTOR AGONISTS ADMINISTERED INTO THE VENTRAL TEGMENTAL AREA ON PHASIC DOPAMINE RELEASE IN RATS

Jakub Duran[1], Jakub Bilnicki[2], Ryszard Bugno[3], Ryszard Przewłocki[4], Wojciech Solecki[1]

[1] *Department Of Neurobiology And Neuropsychology, Institute of Applied Psychology, Faculty of Management and Social Communication, Jagiellonian University, Kraków, Poland*

[2] *Affective Cognitive Neuroscience Laboratory, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[3] *Department Of Medicinal Chemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[4] *Department Of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Opioid analgesics are widely used for the treatment of moderate to severe pain. However, their usage poses a risk of addiction and other adverse effects. The addictive potential of μ-opioid receptor (MOR) agonists is related to their reinforcing effect. This effect is associated with dopamine release, especially phasic release, which has been shown to promote drug seeking. This has led to a search for safer and less addictive opioids. Biased MOR agonists are proposed as such therapeutics due to their diminished side effects. One of such promising opioids is PZM21. In further search for potentially even more potent and safer opioids, PZM21 analogs, like FH210 were created. Subsequently, two novel biased MOR agonists with higher selectivity were developed: BR27 (a PZM21 analog) and BR41 (an FH210 analog).

AIM(S): We aimed to demonstrate the effects of biased MOR agonists on phasic dopamine release in the forebrain.

METHOD(S): We used fast-scan cyclic voltammetry in anesthetized male Sprague-Dawley rats to measure phasic dopamine release evoked by electrical stimulation of the ventral tegmental area. A carbon-fiber microelectrode was inserted into the nucleus accumbens (NAc) and used to measure phasic dopamine release in response to the administration of biased MOR agonists: PZM21, FH210, BR41, BR27.

RESULTS: PZM21, BR41 and BR27 dose-dependently attenuated dopamine release in the NAc, with PZM21 being the most potent. Administration of FH210 also attenuated phasic dopamine release but only at low doses.

CONCLUSIONS: These findings suggest that biased MOR agonists differ in their effects, with a higher selectivity not resulting in higher potency. PZM21 seems to be unique among the tested substances, making it a potential candidate for a safer analgesic with lower addictive potential.

FINANCIAL SUPPORT: NCN, UMO-2020/39/B/NZ7/03537.

## S1P14. LIMITED IMPACT OF ADOLESCENT SOCIAL ISOLATION ON ADULT BEHAVIOUR IN FEMALE C57BL/6J MICE

Magdalena Chrószcz, Klaudia Misiołek, Łukasz Szumiec, Jan Rodriguez Parkitna

*Department of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Social experience during adolescence plays a critical role in shaping adult behaviour. Evidence from both human and rodent studies indicates that social isolation during this period negatively affects emotional regulation and cognitive function. However, it remains unclear whether these impairments reflect permanent alterations or rather may be reversed by later social experience.

AIM(S): This study aimed to assess the effects of social isolation during adolescence, followed by resocialization, on adult social behaviour, anxiety-related responses, and learning.

METHOD(S): Female C57BL/6J mice were isolated for 2 weeks from postnatal days 21 to 35 and subsequently resocialized with same-sex, age-matched peers. Behavioural assessments were conducted on two separate cohorts after 2 or 7 weeks in social housing, using a test battery that evaluated social, emotional, and cognitive domains.

RESULTS: Adolescent isolation had no statistically significant effect on social memory, social rank within the home cage hierarchy, anxiety-like behaviour, or cognitive performance, although a trend toward reduced time spent in the centre of the open field was observed in the younger cohort.

CONCLUSIONS: These findings indicate a mild and potentially transient increase in anxiety-like behaviour, without evidence of broader behavioural deficits. Overall, the data are consistent with the notion that subsequent social experience may buffer the adverse effects of adolescent social isolation. Further studies including both male and female mice are needed to determine the long-term consequences and to better understand potential adaptive responses to adolescent social deprivation.

FINANCIAL SUPPORT: This study was supported by the European Union under the Horizon Europe Framework Programme. Grant Agreement number: E4H/III/4/SI-ADO-AGING/2026.

## S1P15. THE IMPACT OF 1-[(3-FLUOROPHENYL)SULFONYL]-4-(PIPERAZIN-1-YL)-1H-PYRROLO[3,2-C] QUINOLINE (FPPQ) ON MONOAMINE RELEASE IN RAT FRONTAL CORTEX

Agnieszka Wąsik[1], Magdalena Białoń[1], Magdalena Rak[1], Paweł Zajdel[2]

*[1] Department of Neurochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

*[2] Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

INTRODUCTION: Previous studies have shown that FPPQ acts as 5-HT3/5-HT6R antagonists inhibits phencyclidine (PCP)-induced hyperactivity and displaying procognitive properties in the novel object recognition task. So, FPPQ represents a promising approach to respond to the persistent demand for higher efficacy and better compliance in treating drug-resistant schizophrenia symptoms.

AIM(S): The aim of the current study was to measure changes in monoamine (dopamine (DA), noradrenaline (NA), and serotonin (5-HT)) release in the rat frontal cortex following a single intraperitoneal administration of FPPQ (3 mg/kg) alone and in combination with PCP (5mg/kg sc) using the in vivo microdialysis method.

RESULTS: Our results indicated that FPPQ given alone decreased NA and 5-HT release (approx. 10%) in the frontal cortex. At the same time, PCP decreased NA release (approx. 10%). Combined treatment of FPPQ and PCP increased NA and 5-HT release (approx. 25%) in the frontal cortex.

CONCLUSIONS: These results demonstrated that FPPQ, whether administered alone or in combination with PCP, alters the activity of monoaminergic systems in the rat frontal cortex. This alteration may be responsible for the potentially therapeutic effects of FPPQ observed in behavioral tests.

FINANCIAL SUPPORT: The project was financed by the National Science Centre, Poland, no. 2021/43/B/NZ7/02855.

## S1P16. ANTIDEPRESSANT EFFECT OF THE MTOR ACTIVATOR NV-5138 IN AN ANIMAL MODEL OF DEPRESSION: A COMPARISON WITH 7,8-DIHYDROXYFLAVONE

Stanislava Vrankova[1,2], Martina Cebova[1,2], Jana Klimentova[1]

*[1] Centre of Experimental Medicine, Slovak Academy of Sciences, Institute of Normal and Pathological Physiology, Bratislava, Slovakia*

*[2] Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia*

INTRODUCTION: Pathomechanisms involved in the development of depression are mainly associated with the BDNF/TrkB/CREB signaling pathway. Therefore, affecting this pathway is an attractive target for antidepressant therapy and induction of hippocampal neurogenesis. 7,8-dihydroxyflavone (7,8-DHF) is a natural flavonoid that mimics the effects of BDNF. Recent advances showed that mTOR is activated by NV-5138, leading to an amelioration of depressive-like behavior in animals.

AIM(S): The aim of this study was to determine the effect of 7,8-DHF and 7,8-DHF+NV-5138 combination on neural tissue changes related to pathological behavioral symptoms in a chronic unpredictable stress (CUS)-induced model in rats.

METHOD(S): 12-week-old male Sprague Dawley rats were divided into 4 groups: control rats; rats exposed to stressors; CUS-rats that received 7,8-DHF or 7,8-DHF+NV-5138 combination. The rats were administered a single intraperitoneal dose of 7,8-DHF and mTOR activator (NV-5138), 24 hours before behavioural tests. We used forced swim test (FST) to detect behavioral changes. NO-synthase (NOS) activity, protein expression, and concentration of conjugated dienes (CD) were measured.

RESULTS: In FST, we found higher immobility in CUS group. CUS resulted in reduction of NOS activity and decrease in nNOS expression in the medial prefrontal cortex (mPFC). 7,8-DHF decreased immobility in FST and increased NOS activity in mPFC. 7,8-DHF+NV-5138 combination was effective in increasing BDNF and SOD expression in the hippocampus. The concentration of CD was increased after CUS, while 7,8-DHF and combination therapy reduced CD concentration.

CONCLUSIONS: Our results suggest that increased oxidative state in the brain and reduction of NO may play an important role in the pathophysiology of depression. Both 7,8-DHF and NV-5138 were shown to have antidepressant and antioxidant effects. Combination therapy was more effective than monotherapy.

FINANCIAL SUPPORT: The study was supported by grant: VEGA 2/0122/24.

## S1P17. NATURAL ANTIOXIDANTS 7,8-DIHYDROXYFLAVONE AND NARINGIN AS POTENTIAL THERAPEUTIC AGENTS IN EXPERIMENTAL DEPRESSION

Klimentova Jana[1], Ondicova Katarina[1,2], Vrankova Stanislava[1,3]

[1] *Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia*

[2] *Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia*

[3] *Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia*

INTRODUCTION: Naringin and 7,8-dihydroxyflavone (7,8-DHF) are naturally occurring bioflavonoids, that exhibit antioxidant, anti-inflammatory and antidepressant effects. Concurrent administration of 7,8-dihydroxyflavone and Naringin may lead to a synergistic effect, improving NO production and redox status.

AIM(S): The aim of our study was to investigate the effects of 7,8-DHF and Naringin, as well as their combinations on neurobiological and behavioral changes in the model of depression.

METHOD(S): We used 12-week-old female Sprague Dawley rats (SD), which were exposed to unpredictable chronic mild stress (CUS) for 3 weeks. SD rats were divided into five groups: control gorup, CUS group, CUS + 7,8- DHF, CUS + Naringin and CUS + 7,8- DHF + Naringin. We used forced swim test (FST) and elevated plus maze (EPM) test to detect behavioral changes. NO-synthase (NOS) activity, protein expression, and concentration of conjugated dienes (CD) were measured.

RESULTS: We found higher immobility in CUS group in the FST. Both 7,8-DHF and Naringin decreased immobility and the combination was more effective. Rats that were given Naringin or the combination treatment spent more time in the open arms of the EPM and made more entries than the CUS group. NOS activity remained unchanged in the CUS group in medial prefrontal cortex (mPFC). The Naringin and combination of 7,8-DHF+Naringin had significant effect on increasing NOS activity. 7,8-DHF administration increased the expression of nNOS, BDNF, and SOD in the mPFC. The combination of 7,8-DHF+Naringin increased nNOS and SOD1 expression, while Naringin alone increased only SOD1 expression. The concentration of CD was increased after CUS, while 7,8-DHF and 7,8-DHF+Naringin reduced CD concentration.

CONCLUSIONS: Our findings suggested that both 7,8-DHF and Naringin have antidepressant and antioxidant properties, and that combination therapy provides stronger behavioural benefits against changes induced by chronic unpredictable stress.

FINANCIAL SUPPORT: Supported by grant VEGA 2/0122/24.

## S1P18. A MACHINE LEARNING-BASED APPROACH TO ASSESSING THE EFFECTS OF BALANCE AND COGNITIVE-MOTOR TRAINING IN REAL AND EXTENDED ENVIRONMENTS IN SENIORS

Beata Sokołowska[1], Teresa Sadura-Sieklucka[2], Ewa Sokołowska[3]

[1] *Mossakowski Medical Research Institute, Polish Academy of Sciences, Warszawa, Poland*

[2] *National Institute of Geriatrics, Rheumatology and Rehabilitation, Warszawa, Poland*

[3] *Institute of Psychology, John Paul II Catholic University of Lublin, Lublin, Poland*

INTRODUCTION: Computational neuroscience not only bridges the gap between theoretical models and experimental studies to validate them, but also offers advanced computational tools (machine learning and artificial intelligence algorithms, ML/AI) for example, to assess beneficial and adverse effects using highly attractive digital environments such as extended reality (virtual, augmented, and mixed reality, XR: VR/AR/MR). Their application in (neuro)geriatrics is particularly promising, given the rapid aging of modern societies and the serious health consequences associated with it.

AIM(S): The main goal of the research was to evaluate the impact of various training on seniors.

METHOD(S): The study included 76 individuals aged 60 and above who participated in conventional exercises using various equipment (control group) or cognitive-motor exercises using the ActivLife virtual platform (AR group). The results of clinical geriatric tests before and after these exercise sessions were used as learning sets in an ML approach based on the standard k-nearest neighbors (k-NN) decision rule.

RESULTS: After completing the exercise program, both groups demonstrated similar, statistically significant improvements in all clinical tests and a reduced risk of falls. Additionally, participants in the AR group showed greater engagement in the training sessions.

CONCLUSIONS: Unlike traditional cognitive-motor exercise methods, XR technologies with ML/AI offer interactive training, including remote training, and provide feedback on progress and corrected mistakes. This innovative multifaceted approach allows seniors to choose cognitive and motor exercises tailored to their abilities and needs, which they can modify or interrupt

themselves, or continue exercises in a friendlier environment, such as at home. The use of extended environments and advanced computing tools in exercise/training has enormous potential in promoting healthy aging and preventing cognitive-motor function disturbances, as well as balance loss and falls among older adults.

## S1P19. ACCELEROMETRY IS A RELIABLE METHOD FOR DETECTING POSTURAL CHANGES UNDER VIRTUAL HEIGHT EXPOSURE

Zuzana Hirjaková[1], Jana Kimijanová[1], Martin Marko[1,2], Igor Riečanský[1,3], Diana Bzdúšková[1]

*[1] Department of Behavioural Neuroscience, Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia*

*[2] Department of Applied Informatics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Bratislava, Slovakia*

*[3] Department of Psychiatry, Faculty of Medicine, Slovak Medical University in Bratislava, Bratislava, Slovakia*

INTRODUCTION: Wearable inertial sensors are effective, portable and low-cost tools for measuring postural stability. They capture the acceleration of body segments, allowing us to objectively assess postural control via body sway. However, their use for balance assessment at height remains insufficiently investigated, even though height-related situations are common in everyday life. Height exposure can also evoke fear and anxiety, which may modify postural sway.

AIM(S): In this study, we examined whether inertial sensors can sensitively capture postural adjustments associated with height-related fear.

METHOD(S): Forty-two young adults were exposed to virtual heights (0 m, 20 m and 40 m) in an immersive virtual-reality setting, while postural sway was recorded using accelerometers placed on the lower trunk at the level of the fifth lumbar vertebra (L5) and on the upper trunk at the level of the sternum (ST). Participants were divided into two groups: low fear and high fear. To characterize postural sway to each height level and each sensor location, four variables were calculated: 1) root mean square of the acceleration (RMS), 2) mean velocity (MV), 3) mean frequency (MF) and 4) integrated squared jerk (JERK).

RESULTS: Virtual height induced faster and jerkier trunk sway in participants with a high fear of heights, suggesting greater postural stiffening compared with those reporting low fear. Among the sway metrics examined, JERK emerged as the most sensitive and reliable indicator for distinguishing fearful individuals.

CONCLUSIONS: These findings show that accelerometry-based trunk sway assessment can help identify individuals with stability deficits, particularly under challenging conditions. Virtual height exposure revealed clear segmental differences in trunk sway, strong associations with fear of height, and identified JERK as the most sensitive and discriminative measure to distinguish low *vs.* high fear individuals.

FINANCIAL SUPPORT: This work was supported by the Scientific Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic and the Slovak Academy of Sciences (VEGA) grant No. 2/ 0098/25.

## S1P20. MONITORING TRUNK CONTROL AND SITTING STABILITY AFTER STROKE: RESPONSIVENESS OF THE TRUNK IMPAIRMENT SCALE AND THE STROKE IMPACT SCALE TO BIOFEEDBACK-BASED TRAINING

Jana Kimijanová[1], Martin Gábor[2,3], Zuzana Hirjaková[1], Helena Šingliarová[3], Peter Valkovič[1,2], Diana Bzdúšková[1]

*[1] Department of Behavioural Neuroscience, Institute of Normal and Pathological Physiology, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia*

*[2] 2nd Department of Neurology, Faculty of Medicine, Comenius University Bratislava, Bratislava, Slovakia*

*[3] Department of Physical Therapy and Rehabilitation, University Hospital Bratislava, Bratislava, Slovakia*

INTRODUCTION: Stroke survivors frequently exhibit impaired trunk control and reduced sitting stability, which limit mobility, balance, and independence in activities of daily living (ADL). In patients who cannot yet stand safely, sensitive clinical tools are needed to monitor trunk- and sitting-related recovery and to capture intervention-related change.

AIM(S): To evaluate the responsiveness of the Trunk Impairment Scale (TIS) and Stroke Impact Scale (SIS) to a biofeedback-based sensory training program targeting sitting postural stability in subacute stroke patients, and to compare outcomes with standard physiotherapy alone.

METHOD(S): In this randomized controlled study, 40 subacute post-stroke inpatients were allocated to

an Experimental group receiving daily sitting postural training with center-of-pressure–based visual biofeedback in addition to standard physiotherapy, or to a Control group receiving standard physiotherapy only. Trunk control and patient-reported impact were assessed at baseline and post-intervention using the TIS and SIS, including SIS domain scores.

RESULTS: Both groups showed significant improvements over time in trunk control and functional status, reflected by increased TIS and SIS scores. The Experimental group achieved greater gains than the Control group, with the most pronounced effects observed in overall TIS score and in the SIS Mobility and SIS ADL domains. Improvements in mobility were strongly associated with better ADL performance.

CONCLUSIONS: TIS and SIS are responsive to training-related changes following biofeedback-based sensory training in subacute stroke rehabilitation. Complementary trunk-focused intervention with enhanced sensory input yields greater improvements in postural stability and functional mobility than standard therapy alone. Combined use of TIS and SIS provides a clinically meaningful overview of performance-based and patient-reported outcomes relevant to independence after stroke.

FINANCIAL SUPPORT: This work was supported by the Slovak grant agency VEGA No. 2/0098/25 and the Slovak Research and Development Agency under the contract No. APVV-20-0420.

## S1P21. YSR/11-18-BASED ASSESSMENT OF MENTAL AND BEHAVIOURAL PROBLEMS AMONG POLISH ADOLESCENTS

Gabriela Żyłka, Agnieszka Pac

*Chair of Epidemiology and Preventive Medicine, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland*

INTRODUCTION: According to WHO, depression, anxiety and behavioural problems are major causes of illness and disability among adolescents. Recognition of such disorders is crucial for social functioning, physical and mental health, and the ability to lead fulfilling lives.

AIM(S): This study examined the prevalence of mental and behavioural problems in adolescents, with a focus on gender differences.

METHOD(S): The study included data of 168 individuals (56% girls) aged 15-18 years from Kraków, Poland, and the surrounding area, participants of the Kraków birth cohort study. Mental and behavioural problems were assessed using the Youth Self-Report (YSR) for ages 11-18, 2001 version, a widely used self-report measure of adolescent mental health.

RESULTS: The most common problem was anxiety/depression (12.5% of total population), which is a component of the broader internalizing problems that were observed in 10.1% of the total population. Overall, boys exhibited a slightly higher prevalence of mental and behavioural difficulties compared to girls, as they more frequently showed internalizing problems (13.5% of boys *vs.* 7.4% of girls) and externalizing problems (6.8% of boys *vs.* 5.3% of girls). Among boys, the most frequently reported problems were anxiety/depression (20.3%), and, based on DSM-oriented scales, DSM-classified anxiety (14.9%). In the group of girls, the most common issues were withdrawn behaviour (8.5%) and aggressive behaviour (7.4%). Regarding DSM-oriented scales, affective problems were most common among girls (8.5%). Boys had significantly higher prevalence of anxiety/depression (p=0.007) and DSM-classified anxiety (p=0.017) compared to girls.

CONCLUSIONS: These findings highlight the importance of adolescent mental health screening, with attention to gender-specific symptom patterns. Increasing awareness and providing appropriate support may help improve mental health, social functioning, and overall quality of life in late adolescence.

FINANCIAL SUPPORT: The study was funded by project N41/DBS/000725.

## S1P22. BURNOUT SYNDROME IN NEUROSURGEONS: PREVALENCE, DETERMINANTS, AND PREVENTION STRATEGIES

Ram Prasad Subedi[1], Aashish Baniya[1], Bikram Bhandari[1], Suraj Thulung[1], Suresh Bishokarma[1], Sagun Ghimire[2]

*[1] Department of Neurosurgery, Upendra Devkota Memorial National Institute of Neurological and Allied Sciences, Bansbari, Kathmandu, Nepal*

*[2] Department of Surgery, B and B Hospital, Gwarko, Lalitpur, Nepal*

INTRODUCTION: Burnout syndrome (BS), defined by emotional exhaustion, depersonalization, and reduced personal accomplishment, is increasingly recognized in neurosurgery, a specialty marked by high cognitive demand, prolonged operative hours, medico-legal pressure, and intense academic expectations. Beyond

personal distress, burnout affects patient safety, clinical outcomes, and workforce sustainability.

AIM(S): To examine the global prevalence of burnout in neurosurgeons and trainees, analyze its determinants using an ecological framework, and outline evidence-based prevention strategies.

METHOD(S): A structured narrative review was conducted using literature from PubMed, Scopus, and Google Scholar with keywords including "neurosurgeon burnout" and "occupational stress." Evidence from surveys, meta-analyses, and interventional studies was synthesized. Determinants were categorized into macro-(healthcare systems), meso-(institutional culture), and micro-level (individual factors) domains.

RESULTS: Reported burnout prevalence ranges from approximately 48–67% among practicing neurosurgeons, with pooled estimates near 51% among residents. Macro-level drivers include administrative workload, electronic documentation burden, productivity-based economic models, and medico-legal stress. Meso-level factors involve leadership style, staffing adequacy, operational inefficiencies, and cultural stigma toward mental health. Micro-level contributors include perfectionism, maladaptive coping, sleep deprivation, and poor work–life integration. Burnout is associated with depression, substance misuse, cardiovascular risk, increased medical errors, reduced empathy, lower patient satisfaction, and workforce attrition. Multi-level interventions such as EHR optimization, rationalized duty hours, supportive leadership, peer-support systems, and mindfulness-based programs demonstrate greater effectiveness when implemented collectively.

CONCLUSIONS: Burnout in neurosurgery represents a systemic challenge rather than individual weakness.

FINANCIAL SUPPORT: Self-supported.

## S1P23. EFFICACY OF DEXMEDETOMIDINE IN PREVENTING POSTOPERATIVE DELIRIUM IN PATIENTS UNDERGOING BRAIN SURGERY: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMISED CONTROLLED TRIALS

Eloísa Bittencurt Thomaz de Assis[2], Maria Angélica Otero de Melo dos Reis[1], Harshada Kalaiarasan Swamy[2], Baltazar César Miranda Matos[1], Marcio Yuri Ferreira[3], Netanel Ben-Shalom[3], Juliana Estima Rissi Polverini[4], Allan Dias Polverini[5]

[1] *Faculty of Medicine, National University of Rosario, Rosario, Argentina*
[2] *Faculty of Medicine, Baltic Federal University, Kaliningrad, Russian Federation*
[3] *Department of Neurosurgery, Northwell Health Hospital, New York, United States of America*
[4] *Department of Anaesthesiology, Barretos Cancer Hospital, São Paulo, Brazil*
[5] *Neurosurgical Oncology Division, Barretos Cancer Hospital, São Paulo, Brazil*

INTRODUCTION: Dexmedetomidine (DEX) is a selective α2-adrenergic receptor agonist widely used for its sedative and neuroprotective properties. Patients undergoing brain surgery are at increased risk of postoperative delirium (POD), a complication associated with poorer neurological outcomes.

AIM(S): To evaluate the efficacy and safety of DEX for POD prevention in adult neurosurgical patients through a systematic review and meta-analysis.

METHOD(S): PubMed, Embase, Scopus, and Web of Science were searched from inception to January 2025 and updated in April 2025, in accordance with PRISMA guidelines. Randomised controlled trials assessing DEX for POD prevention in adult patients undergoing brain surgery were included. Risk of bias was evaluated using standard tools, and meta-analysis was performed using Review Manager 5.4.1. The review was prospectively registered in PROSPERO (CRD42025649703).

RESULTS: Five randomised controlled trials comprising 752 adult patients were included. DEX was administered with a loading dose of 0.5–1 μg/kg over 10 minutes, followed by maintenance infusions of 0.1–0.5 μg/kg/hour, delivered intraoperatively and/or during the early postoperative period. DEX significantly reduced the incidence of POD (RR 0.47; 95% CI 0.35–0.63; $p<0.00001$), representing a 53% relative risk reduction, with no heterogeneity ($I^2=0\%$). In subgroup analyses, brain tumour resection (n=319) showed a comparable reduction (RR 0.47; 95% CI 0.33–0.68; $p<0.0001$). Similar effects were observed in mixed cranial surgery populations (n=322; RR 0.47; 95% CI 0.29–0.76; p=0.002), also without heterogeneity. Adverse events were comparable between groups and were generally mild and manageable with standard interventions.

CONCLUSIONS: DEX was associated with a consistent and clinically meaningful reduction in POD in adult patients undergoing brain surgery. Further studies are required to define optimal dosing strategies and the timing of administration.

## S1P25. TARGETED BLOCKING OF GENE SPLICING CAN DYSREGULATE INTRON-EMBEDDED PRIMARY MICRORNAS AND AXONAL MORPHOGENESIS

Md Hasan Ali[1], Athul R. Ramesh[1], Naveen Nedunchezhian[1], Wojciech Kwiatkowski[1], Piotr Kopeć[1], Natalia Kowalewska[1], Sebastian Pęcherz[1], Barbara Gnutti[2], Dario Finazzi[2], Savani Anbalagan[1]

[1] *Institute of Molecular Biology & Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland*

[2] *Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy*

INTRODUCTION: Addressing whether antisense oligonucleotide (ASO)-based targeting of neuroglial genes embedded with intronic noncoding RNAs (ncRNAs) affects the expression and function of intronic ncRNAs is crucial for ASO success in clinical trials. In vitro studies are limited in their ability to assess ASO toxic effects.

AIM(S): The aim of this study was to determine whether ASOs targeting the slit3 gene in zebrafish affect slit3 intron-embedded mir-218a-1 expression or function in neurohypophyseal axonal morphogenesis.

METHOD(S): Wildtype or mir218a-1 mutant zebrafish single-cell stage embryos were injected with control ASOs or ASOs targeting slit3 splice sites or mir-218a-1-adjacent splice sites or mir-218a-1 mimics. Zebrafish larvae were imaged on confocal microscopy and neurohypophyseal axonal morphogenesis was quantified.

RESULTS: We observed that an ASO targeting the slit3 splice site but not the one targeting its translation start site, disrupts neurohypophyseal axonal morphogenesis. In addition to altered slit3 splicing, we also observed increased expression of slit3 and slit3 intron-embedded primary mir218a-1 transcripts. The ASO-induced phenotype was not observed when mature mir218a-1 was blocked by an ASO or in mir218a-1-/- mutants. The axonal phenotype was also phenocopied in samples injected with mir-218a mimic in a dose-dependent manner.

CONCLUSIONS: Our results indicate that despite ASO-induced genetic compensation, intron-retained transcripts can escape NMD machinery, become stabilized, and drive increased intronic primary microRNA expression and function. As prematurely terminated intron-retained transcripts can translocate to axons and impact neuronal function, our study warrants further validation across ncRNA classes.

FINANCIAL SUPPORT: National Science Centre grant [SONATA 2021/43/D/NZ3/01798].

## S1P26. DECODING THE GLYCO-LANDSCAPE: ANALYSIS OF A-2.6 SIALYLATION PATTERNS IN ASTROCYTE AND MICROGLIAL CELL LINES

Alicja Mejza, Bożena Szulc

*Department of Biochemistry, Faculty of Biotechnology, University of Wrocław, Wrocław, Poland*

INTRODUCTION: Sialylation is a post-translational modification of molecules by adding sialic acid. Dysregulated sialylation influences the immune response and is observed in diseases such as Multiple Sclerosis (MS). Astrocytes and microglia regulate de- and remyelination, and dysregulated activation of these cells drives MS progression. Our unpublished data show that especially α-2.6 sialylation increases in astrocytes and microglia in the cuprizone mouse model of MS.

AIM(S): This study aims to characterize the α-2.6 sialylation profiles of astrocyte and microglial cell lines, with a primary interest in differences in sialylation patterns that may explain molecular mechanisms of glial dysregulation in MS pathology.

METHOD(S): We used murine BV-2 (microglia) and C8-D1A (astrocytes) cells. α-2.6 sialylation was assessed with Sambucus nigra agglutinin – α2,6-linked sialic acids binding protein (SNA lectin). Comparative α-2.6 sialylation patterns between BV-2 and C8-D1A cells were evaluated using immunofluorescence staining. We also compare, by Western blotting, protein levels of enzymes involved in sialic acid metabolism, including sialyltransferase ST6GAL1 and neuraminidase 1 (NEU1). Intracellular localization of those proteins was characterized using specific antibodies and high-resolution confocal microscopy.

RESULTS: Both BV-2 and C8-D1A cells showed prominent α2,6-sialylation. NEU1 was strongly expressed and localized at the cell membrane surface, but also intracellular in the lysosomes. Sialyltransferase ST6GAL1 was observed in the Golgi apparatus, with no differences in the protein level between glial cells. We also observed that most of α2,6-sialylated proteins are localized mainly in the Golgi apparatus.

CONCLUSIONS: Integrating glycobiology with glial biology, this study highlights the role of glycosylation in glial function and suggests mechanisms underlying neuroinflammation and neurodegeneration. These cells provide suitable in vitro models for studying cell-specific glycosylation and its functional consequences.

FINANCIAL SUPPORT: Research funded by the Department of Biochemistry, Faculty of Biotechnology at the University of Wrocław.

## S1P27. ANALYSIS OF DENDRITIC SPINE MORPHOLOGY UNDER MATERNAL HIGH-FAT DIET AND SHORT-CHAIN FATTY ACID TREATMENT

Mateusz Sar, Magdalena Rak, Natalia Parzych, Anna Wesołowska, Kinga Gawlińska

*Department of Clinical Pharmacy, Faculty of Pharmacy, Jagiellonian University Collegium Medicum, Kraków, Poland*

INTRODUCTION: A high-fat diet modulates the composition of the gut microbiota, which affects the susceptibility of the offspring to the development of neurodevelopmental disorders. A particularly important role is played by the maternal high-fat diet (mHFD), which is associated with an increased risk of conditions such as autism spectrum disorder (ASD) in the offspring. Changes occurring in the offspring's brain may be mediated by the gut–brain axis and by microbiota-derived metabolites, including short-chain fatty acids (SCFAs). These mechanisms may lead to metabolic disturbances as well as modifications in dendritic spine morphology.

AIM(S): The aim of this study was to investigate the effects of maternal high-fat diet and SCFA supplementation on the morphology of dendritic spines in cortical neurons. Maternal metabolic parameters, including body weight and consumption-related measures, were monitored during the dietary intervention.

METHOD(S): C57BL/6J female mice were used as the experimental model. Four experimental groups were established: animals receiving a control diet (CD; 10% kcal from fat) with water or a 1 mM sodium butyrate (SB) solution as the SCFA, and animals fed a high-fat diet (HFD; 45% kcal from fat) with water or 1 mM SB. To compare dendritic spine morphology, primary cortical neurons were cultured from the cerebral cortex of embryos at embryonic day 18.5 (E18.5). On day 18 in vitro, the cells were incubated for 48 hours with different SCFAs: sodium acetate (SA, 1 mM), SB (1 mM), sodium propionate (SP, 1 mM), or a mixture of all three (1 mM of each compound). Neurons were visualized using the DiI dye, and imaging was performed with confocal microscopy.

RESULTS: Preliminary observations suggest that SCFA treatment may modulate dendritic spine morphology. Maternal diet was also associated with measurable alterations in metabolic parameters.

CONCLUSIONS: The obtained results may suggest that the use of a different diet and SCFA may lead to changes in the dendritic spine morphology and dietary patterns.

FINANCIAL SUPPORT: The study was supported by research grant SONATA no. 2023/51/D/NZ5/02090 from the National Science Centre (Kraków, Poland).

## S1P28. EVOLUTION AND DEVELOPMENT OF THE HUMAN BRAIN THROUGH THE LENS OF PROTEIN N-GLYCOSYLATION

Thomas S. Klarić[1,2], Ivan Gudelj[1,2,3], Gabriel Santpere[1,4], Mislav Novokmet[2], Frano Vučković[2], Shaojie Ma[1], Hannah M. Doll[5,6], Ryan Risgaard[5,6], Shveta Bathla[7], Amir Karger[8], Angus C. Nairn[7], Victor Luria[1,9], Ivona Bečeheli[2], Chet C. Sherwood[10], John J. Ely[11,12], Patrick R. Hof[13], André M. M. Sousa[5,6], Djuro Josić[3,14], Gordan Lauc[2,15], Nenad Sestan[1,7,16]

[1] *Department of Neuroscience, Yale School of Medicine, New Haven, CT, USA*
[2] *Genos Glycoscience Research Laboratory, Zagreb, Croatia*
[3] *Department of Biotechnology, University of Rijeka, Rijeka, Croatia*
[4] *Hospital del Mar Research Institute, Barcelona, Catalonia, Spain*
[5] *Waisman Center and Department of Neuroscience, School of Medicine and Public Health, University of Wisconsin Madison, Madison, WI, USA*
[6] *Department of Neuroscience, University of Wisconsin Madison, Madison, WI, USA*
[7] *Department of Psychiatry, Yale School of Medicine, New Haven, CT, USA*
[8] *Harvard Medical School, IT Research Computing, Boston, MA, USA*
[9] *Boston Children's Hospital, Division of Genetics and Genomics, Harvard Medical School, Boston, USA*
[10] *Department of Anthropology, The George Washington University; Washington, District of Columbia, USA*
[11] *Center for the Advanced Study of Human Paleobiology, The George Washington University; Washington, District of Columbia, USA*
[12] *MAEBIOS, Alamogordo, NM, USA*
[13] *Nash Family Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai; New York, NY, USA*
[14] *Warren Alpert Medical School, Brown University, Providence, RI, USA*
[15] *University of Zagreb Faculty of Pharmacy and Biochemistry, Zagreb, Croatia*
[16] *Departments of Genetics and Comparative Medicine, Kavli Institute for Neuroscience, Program in Cellular Neuroscience, Neurodegeneration and Repair, and Yale Child Study Center, Yale School of Medicine, New Haven, CT, USA*

INTRODUCTION: Although numerous comparative "omics" studies have revealed unique aspects of human neurobiology, the contribution of protein glycosylation (post-translational sugar modifications) has not been extensively explored. Likewise, little is known about the trajectory of the brain's glycome during human neurodevelopment.

AIM(S): To identify human-specific features of brain N-glycosylation and to characterize the spatiotemporal dynamics of N-glycosylation during human brain development.

METHOD(S): We performed multi-regional characterization of rat, macaque, chimpanzee, and human brain N-glycomes using chromatography and mass spec-

trometry, then integrated these data with complementary bulk tissue and single-nucleus glycotranscriptomic data. We also performed a multi-regional comparison of the prenatal and adult human brain N-glycomes.

RESULTS: In primates, the brain N-glycomes have diverged more rapidly than the underlying transcriptomic frameworks, which implies that N-glycosylation provides a means for rapidly generating interspecies diversity at the molecular level. We uncovered numerous phylogenetic trends in brain protein N-glycosylation as well as several human-specific adaptations. Moreover, interspecies differences in the cell type expression pattern of key glycogenes were identified, including some human-specific differences, which may underpin this evolutionary divergence. Regarding glycosylation during human brain development, we uncovered both global and region-specific N-glycome maturation pathways that together lead to distinct neuroanatomical N-glycosylation profiles and thus contribute to the spatial molecular diversity of the mature brain.

CONCLUSIONS: Brain N-glycome evolution in hominids has been characterized by an overall increase in complexity coupled with a shift toward increased usage of α(2-6) linked N-acetylneuraminic acid. Also, region-specific neurodevelopmental pathways lead to distinct spatial N-glycosylation profiles in the mature brain.

FINANCIAL SUPPORT: NIH grants MH124619, MH116488, DA053628, and R01HG010898-01. Instituto de Salud Carlos III grant MS20/00064. Agencia Estatal de Investigación grant PID2019-104700GA-I00/AEI/10.13039/501100011033. The European Union Seventh Framework Programme (FP7 2007 2013) under grant agreement no. 291823 Marie Curie FP7-PEOPLE-2011-COFUND (The New International Fellowship Mobility Programme for Experienced Researchers in Croatia NEWFELPRO). We acknowledge the National Chimpanzee Brain Resource (funded by NIH grant NS092988) for contributing brain specimens used in this study.

## S1P29. SPHEROID MODEL TO STUDY NEURON-OLIGODENDROCYTE INTERACTIONS

Maciej Degutis, Katarzyna Starowicz-Bubak, Katarzyna Popiołek-Barczyk

*Department of Neurochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: In the mammalian brain, neural stem cells (NSCs) reside in specialized microenvironments within the subgranular zone (SGZ) and subventricular zone (SVZ), where they possess distinct differentiation potentials. Three-dimensional neural spheroid and organoid models differ in their degree of tissue self-organization. Organoids exhibit complex regional architecture, whereas spheroids provide uniform and reproducible cellular assemblies that are particularly well suited for controlled intervention studies and the dissection of specific cell-to-cell interaction mechanisms.

AIM(S): Here, we developed a 3D neural spheroid-based model derived from primary mouse postnatal NSCs undergoing multilineage differentiation, resulting in functional neuronal networks and myelination.

METHOD(S): Spheroids were generated from primary mouse postnatal SVZ- and SGZ-derived NSCs to assess niche-specific differentiation potential. Immunocytochemical analysis was used to confirm the presence of mature astrocytic, neuronal, and oligodendrocytic markers. Neuronal activity was assessed using calcium imaging in response to glutamate and histamine.

RESULTS: Immunocytochemical staining revealed the colocalization of myelin sheets with axons and the maintenance of niche-dependent lineage marker expression. Calcium imaging revealed functional neuronal network activity patterns and propagating astrocytic calcium waves across spheroids, indicative of network-level glial signalling.

CONCLUSIONS: This platform enables the investigation of cell-to-cell interaction and communication mechanisms that are inaccessible in vivo or oversimplified in 2D in vitro systems, providing a simple yet reproducible tool for both basic and applied research.

FINANCIAL SUPPORT: Work was financed by the National Science Centre, Poland, 2025/57/N/NZ5/03530, and 2021/43/B/NZ7/01162.

## S1P30. EVALUATION OF MATERNAL HIGH-FAT DIET AND SODIUM BUTYRATE SUPPLEMENTATION ON THE NEUROINFLAMMATORY PROFILE OF OFFSPRING CEREBELLUM

Magdalena Rak, Natalia Parzych, Kinga Gawlińska, Anna Wesołowska

*Faculty of Pharmacy, Department of Clinical Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

INTRODUCTION: Maternal nutrition is a key determinant of offspring neurodevelopment. Investigating the impact of a mother's diet on her offspring will open up new possibilities for understanding developmental diseases in offspring and will also aid in preventative measures.

AIM(S): This study evaluated the effects of a maternal high-fat diet (HFD) and sodium butyrate (SB) supplementation during pregnancy and lactation on pro-inflammatory cytokines in the mouse offspring cerebellum.

METHOD(S): Female C57BL/6J mice were fed a control diet (CD, 10% energy from fat) or an HFD (45% energy from fat) for 8 weeks before mating. After conception, a subset of HFD-fed mice was supplemented with sodium butyrate (HFD+SB) in drinking water during pregnancy and lactation. Cerebellar samples were collected from male and female offspring at postnatal days (PND) 14 and 42. Cytokine levels (IL-1β, IL-6, TNF-α) as well as total level of short-chain fatty acids (SCFAs) were measured using ELISA.

RESULTS: No significant differences in the protein levels of IL-1β or TNF-α were observed between groups at PND 14 or PND 42. The protein level of IL-6 was reduced in the cerebellum of female offspring from the HFD+SB group at PND 14. Additionally, no changes in cerebellar SCFA levels were detected at the evaluated time points.

CONCLUSIONS: In conclusion, maternal HFD did not induce marked cerebellar neuroinflammatory alterations during early postnatal development. However, SB supplementation during pregnancy and lactation may reduced IL-6 levels in female offspring, suggesting potential sex-specific protective effects. Further studies are needed to assess the long-term neuromodulatory effects of maternal HFD and SCFA supplementation and their impact on offspring neurodevelopment.

FINANCIAL SUPPORT: This work was supported by statutory funds of the Department of Clinical Pharmacy, Faculty of Pharmacy, JU CM (N42/DBS/000403) and the National Science Centre (SONATA grant no. 2023/51/D/NZ5/02090).

## S1P31. EFFECT OF KETOGENIC DIET ON HYPPOCAMPAL ASTROCYTES

Nina Prokopek, Joanna Jędrusik, Zuzanna Setkowicz

*Laboratory of Experimental Neuropathology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*

INTRODUCTION: The ketogenic diet (KD) is a high-fat, low-carbohydrate nutritional regimen with moderate protein content that induces a metabolic state of ketosis, in which fats replace glucose as the primary energy source. Over the past century, the therapeutic role of KD in the treatment of epilepsy has been widely recognized. However, its impact on early development of nervous system remains unknown. Given the rising non-medical popularity of KD and its increasing use among healthy individuals, it is essential to evaluate its potential adverse consequences. Hippocampal astrocytes play essential roles in brain development, including participation in neurogenesis and ketone body transport across the blood–brain barrier (BBB).

AIM(S): The aim of the present study was to evaluate the effects of KD on hippocampal astrocytes during nervous system development using a rat model.

METHOD(S): Pregnant Wistar females were divided according to diet into a ketogenic diet group (KD) and a normal diet group (ND), as well as a group in which the diet was switched from KD to ND on the second postnatal day (KDND). On postnatal day 60 (P60), offspring were perfused, and their brains were collected, sectioned, and processed using immunohistochemical staining technique.

RESULTS: To assess astrocytic reactivity within the hippocampus, GFAP immunostaining was performed followed by quantitative microscopic analysis. Based on acquired hippocampal images, the immunoreactive area of GFAP-positive cells was quantified using ImageJ.

CONCLUSIONS: By examining how maternal ketogenic diet influences astrocyte development, this study addresses an important gap in our understanding of the diet's potential impact on the maturation of the offspring's nervous system.

## S1P32. EFFECTS OF MATERNAL GLP-1 RECEPTOR AGONIST EXPOSURE ON EARLY NEUROLOGICAL DEVELOPMENT IN MOUSE OFFSPRING

Wiktoria Płonka, Bernadeta A. Pietrzak-Wawrzyńska, Andrzej Łach, Agnieszka Wnuk

*Team III, Department of Pharmacokinetics and Drug Metabolism, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Semaglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist, widely used in the treatment of diabetes and obesity. Despite its increasing clinical use, limited data exist regarding the effects of maternal semaglutide exposure during pregnancy on offspring development. Manufacturers recommend discontinuing therapy two months before a planned pregnancy, yet nearly 45% of pregnancies are unplanned and detected in the first trimester, leading to continued treatment in early pregnancy.

AIM(S): This study aimed to evaluate the effects of maternal semaglutide exposure during pregnancy on early offspring development.

METHOD(S): Female Swiss albino mice received increasing doses of semaglutide or saline prior to mating and throughout 14 days of pregnancy. Early offspring development was evaluated through developmental milestones tests. Observations were conducted daily from day 3 to day 14 of pup life. The analyzed key milestones included: pinnae detachment (ear unfolding), ear opening, eye opening, fur development, crawling, transition from crawling to walking. These developmental markers reflect somatic and neuromotor maturation during early life.

RESULTS: Offspring of semaglutide-treated mothers demonstrated delayed timing of selected developmental milestones. Eye opening and ear opening occurred later compared to control, with the most pronounced differences observed on postnatal day 13. In contrast, motor development analysis revealed earlier onset of walking in offspring prenatally exposed to semaglutide, with significant differences also noted on 13 PND. No marked differences between groups were observed in the remaining milestones (pinnae detachment, fur development, crawling, transition).

CONCLUSIONS: These results suggest that maternal semaglutide exposure may modulate the pace of early neurological and motor development in offspring, delaying sensory maturation while accelerating locomotor development, highlighting the need for further studies on prenatal GLP-1 agonist exposure.

FINANCIAL SUPPORT: National Science Centre of Poland, grant number 2023/51/B/NZ7/02360 and statutory fund of IP PAS.

## S1P33. TREADMILL EXERCISE RESHAPES THE HIPPOCAMPAL PROTEOME IN PRENATALLY DEXAMETHASONE-EXPOSED RATS

Katarzyna Głombik[1], Magdalena Kukla-Bartoszek[1], Maciej Suski[2], Katarzyna Curzytek[1], Beata Grygier[1]

*[1] Laboratory of Immunoendocrinology, Department of Experimental Neuroendocrinology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

*[2] Department of Pharmacology, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland*

INTRODUCTION: Prenatal exposure to synthetic glucocorticoids like dexamethasone can lead to long-term neurobiological programming and increased susceptibility to stress-related disorders.

AIM(S): We aimed to evaluate whether regular physical activity could serve as a protective strategy to modulate the hippocampal proteome and mitigate the biochemical abnormalities induced by such early-life stress.

METHOD(S): Pregnant Sprague Dawley rats received subcutaneous dexamethasone (0.1 mg/kg) from gestational day 14 until delivery to induce depressive-like behavior in offspring. At four weeks of age, male offspring underwent a 4-week treadmill exercise program consisting of 30-minute daily sessions at speeds up to 8 m/min, 5 days per week, to assess the impact of physical training on the hippocampal proteome. Proteomic profiling was performed using SWATH-MS, a data-independent acquisition (DIA) quantitative proteomic approach.

RESULTS: Proteomic analysis revealed that treadmill exercise significantly regulated the expression of 109 proteins in the hippocampus of rats prenatally exposed to dexamethasone. Among these differentially expressed proteins, 26 showed significant up-regulation, while 83 were significantly decreased following the training intervention. The exercise-induced up-regulation of NQO2 suggests an enhanced neuroprotective and antioxidant response, which, combined with the down-regulation of Tyrosine 3-monooxygenase and CRTC2, points to a comprehensive normalization of hippocampal redox balance and catecholamine signaling.

CONCLUSIONS: Our findings demonstrate that moderate treadmill exercise can normalize the neurobiological programming induced by prenatal dexamethasone exposure by modulating the hippocampal proteome, specifically enhancing NQO2-mediated neuroprotection and normalizing catecholamine and metabolic signaling by down-regulating Tyrosine 3-monooxygenase and CRTC2.

FINANCIAL SUPPORT: This work was supported by grant 2020/39/D/NZ7/01610 from the National Science Centre, Poland.

## S1P34. EARLY EXPOSURE TO A KETOGENIC DIET MAY ADVERSELY INFLUENCE BRAIN DEVELOPMENT AND INDUCE ALTERATIONS IN THE GUT MICROBIOTA COMPOSITION

Joanna Jędrusik[1,2], Michał Kobiałka[1], Zuzanna Bilnicka[1], Anna Grzesiak[1], Zuzanna Setkowicz[1]

[1] *Laboratory of Experimental Neuropathology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*

[2] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: The ketogenic diet (KD), a highfat, lowcarbohydrate dietary intervention modifies the gut microbiota, one of a key regulator of early brain development.

AIM(S): This study explored how prenatal and early postnatal KD exposure influences overall brain and body growth and alters offspring gut microbiota at postnatal days (P) 2, 30, and 60.

METHOD(S): Wistar rats were assigned to a normal diet (ND), KD during gestation and lactation, or prenatal KD followed by ND after birth (KD/ND). Entire intestines were collected at P2, and caecal samples at P30 and P60. Microbiota composition was assessed by 16S rDNA sequencing (Illumina). Body and brain mass were recorded at a perfusion day (P30, P60).

RESULTS: Microbiota composition differed across diets and ages, with the strongest shifts observed in KDexposed P2 pups. In P2 animals, KD reduced Proteobacteria and increased Actinobacteria. At P30, KD elevated Bacteroidota and Firmicutes and decreased Actinobacteria. Changes were normalized by postnatal switch to ND. At P60, KD increased Proteobacteria, Bacteroidota, Firmicutes, and Actinobacteria, while ND introduction partially reversed these effects. KDexposed animals displayed significantly reduced body and brain mass compared with ND and KD/ND groups, while braintobody ratios were higher in KD and KD/ND animals.

CONCLUSIONS: Early KD exposure induces alterations general brain development and in gut microbiome structure. Such changes can be partially normalized by a standard diet introduction after birth. These findings highlight the potential role of microbiome-mediated mechanisms in KD-induced effects on neurodevelopment. It provides a strong foundation for investigating the extent to which KD affects the developing brain.

FINANCIAL SUPPORT: N/A.

## S1P35. THE ROLE OF MATERNAL HIGH-FAT DIET AND PROBIOTIC SUPPLEMENTATION DURING PREGNANCY AND LACTATION IN SHAPING BEHAVIORAL PHENOTYPES OF MOUSE OFFSPRING: A POTENTIAL ROLE OF THE GUT–BRAIN AXIS

Nataliia Kolach[1], Natalia Malikowska-Racia[2], Renata Pieniążek[1], Dawid Gawliński[1]

[1] *Department of Drug Addiction Pharmacology, Maj Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland*

[2] *Department of Behavioral Neuroscience & Drug Development, Maj Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Maternal obesity induced by a high-fat diet (HFD) has been shown to adversely affect offspring brain development, increasing vulnerability to psychiatric and neurodevelopmental disorders. Exposure to a maternal high-fat diet (mHFD) may also alter offspring gut microbiota composition, thereby disrupting gut–brain axis signaling.

AIM(S): The aim of this study was to assess behavioral outcomes and gut microbiota composition in offspring exposed to mHFD, as well as to evaluate the efficacy of probiotic supplementation during pregnancy and lactation in mitigating mHFD-induced adverse effects.

METHOD(S): Female C57BL/6J mice were fed either a control diet (10% of energy from fat) or an HFD (45% of energy from fat) for 8 weeks prior to mating and throughout pregnancy and lactation. A subset of HFD-fed dams received a probiotic (Lacticaseibacillus rhamnosus and Lactobacillus helveticus) in drinking water ($10^9$ colony-forming units/mL) during pregnancy and lactation. Offspring ultrasonic vocalizations were assessed during infancy, followed by behavioral testing in adolescence, including the open field test, self-grooming test, marble burying test, elevated plus maze (EPM), and novel object recognition test. Gut microbiota composition was analyzed in offspring fecal samples using shotgun metagenomic sequencing.

RESULTS: Notably, mHFD significantly reduced the time spent by female offspring in the open arms of the EPM, an effect reversed by maternal probiotic supplementation. Furthermore, mHFD decreased the Shannon diversity index in offspring of both sexes, whereas probiotic supplementation restored microbial diversity in female offspring to levels comparable to controls.

CONCLUSIONS: In conclusion, mHFD may predispose female offspring to anxiety-like behaviors and induce gut microbiota dysbiosis in a sex-independent manner. Maternal probiotic supplementation during exposure to HFD may protect against behavioral disturbances and partially normalize gut–brain axis function in offspring.

FINANCIAL SUPPORT: This study was supported by a grant no. 2021/43/B/NZ5/02552 from the National Science Centre (Poland).

## S1P36. PRENATAL ANTIBIOTICS INDUCED DYSBIOSIS INDUCES PERSISTENT HIPPOCAMPAL MICROGLIOSIS AND ANXIETY-LIKE BEHAVIOR IN OFFSPRING

Rishikesh[1], Dayamrita K K[1], Krishnapriya[1], Ayswaria Deepti[1], Aneesa P A[1], Baby Chakrapani P S[1,6], Shubham Upadhyay[2], Keshava Prasad T S[2], Unnikrishnan Sivan[3], Isabell Haack[4,5], Shirin Hosseini[4,5], Martin Korte[4,5]

[1] *Centre for Neuroscience, Department of Biotechnology, Cochin University of Science and Technology (CUSAT), Kochi, Kerala, India*

[2] *Centre for Systems Biology and Molecular Medicine, Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore, India*

[3] *Department of FSQA, FFE, Kerala University of Fisheries and Ocean Studies (KUFOS), Panangad, Kochi, Kerala, India*

[4] *Department of Cellular Neurobiology, Zoological Institute, Technical University Braunschweig, Germany*

[5] *Helmholtz Centre for Infection Research, Neuroinflammation and Neurodegeneration Group, Braunschweig, Germany*

[6] *Centre of Excellence in Neurodegeneration and Brain Health, Cochin University of Science and Technology (CUSAT), Kochi, Kerala, India*

INTRODUCTION: Maternal gut dysbiosis during pregnancy influences offspring neurodevelopment via immune, metabolic, and microglial pathways. While severe disruptions are known to affect brain maturation, the impact of short-term antibiotic exposure remains unclear. Even subtle microbiota perturbations may alter neuroimmune programming, potentially increasing long-term behavioral vulnerability.

AIM(S): This study aimed to determine whether transient maternal antibiotic exposure during pregnancy alters maternal and offspring gut microbiota and programs persistent neurobehavioral and neuroimmune alterations in adolescent offspring.

METHOD(S): Pregnant dams received an oral antibiotic cocktail for 7 days to induce gut microbiota perturbation. Maternal and offspring phenotypes were assessed using 16S rRNA sequencing, behavioral testing (Elevated Plus Maze, Y-maze, Forced Swim Test, sociability and recognition paradigms), peripheral immune profiling, cytokine analysis, metabolomics, and hippocampal immunohistochemistry at postnatal day 49.

RESULTS: Maternal antibiotic exposure did not alter microbial diversity, maternal care, intestinal integrity, HPA-axis function, or developmental milestones, though offspring ASV distribution differed. Adolescent offspring displayed anxiety-like behavior and cognitive deficits despite intact recognition memory and sociability. Peripheral immune cells (WBCs, neutrophils) were elevated without changes in cytokines or BDNF. Hippocampal analysis revealed marked microgliosis, enhanced synaptic engulfment, and astrocytic reactivity.

CONCLUSIONS: Transient maternal antibiotic exposure, even without major microbiome shifts, triggers lasting neuroimmune remodeling in offspring—characterized by microglial activation, synaptic pruning, peripheral immune changes, and anxiety-like behaviors. The findings underscore the developing brain's sensitivity to subtle maternal gut perturbations and the need for judicious antibiotic use during pregnancy.

FINANCIAL SUPPORT: Funding from project.

## S1P37. PROTECTING THE BRAIN BY STRENGTHENING BIOLOGICAL BARRIERS: YOLKIN AND OVOCYSTATIN AS REGULATORS OF THE GUT–BRAIN AXIS

Agnieszka Zabłocka[1], Bartłomiej Stańczykiewicz[2], Dominika Jakubczyk[1], Sabina Górska[1], Krzysztof Gołąb[3], Józefa Macała[1], Błażej Misiak[2]

[1] *Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Laboratory of Microbiome Immunobiology, Wrocław, Poland*

[2] *Division of Consultation Psychiatry and Neuroscience, Department of Psychiatry, Wrocław Medical University, Wrocław, Poland*

[3] *Department of Pharmaceutical Biochemistry, Division of Pharmaceutical Biochemistry, Wrocław Medical University, Wrocław, Poland.*

INTRODUCTION: The integrity of biological barriers within the gut–brain axis, including the intestinal epithelium (IE) and brain microvascular endothelium (BME), is crucial for maintaining brain homeostasis. Barrier disruption and increased permeability are linked to neuroinflammation and impaired brain function. Egg-derived proteins, such as the yolkin polypeptide complex and ovocystatin, show immunomodulatory and protective effects, yet their impact on gut and brain barriers is not fully understood.

AIM(S): To evaluate whether yolkin and ovocystatin can support intestinal and blood–brain barrier integrity and function.

METHOD(S): Yolkin and ovocystatin were isolated from hen eggs. HT29 and HBEC5i cell lines modelled intestinal and brain barriers. Cell viability and cytotoxicity were assessed via MTT and LDH assays. Barrier integrity was analyzed using TEER and paracellular permeability measurements. Secretion of pro- and anti-inflammatory mediators was measured with ELISA and multiplex immunoassays.

RESULTS: Neither protein affected viability or induced cytotoxicity. Both modulated barrier integrity and permeability under normal and inflammatory conditions, with distinct patterns. Yolkin and ovocystatin selectively stimulated IL-1β and IL-8 secretion. Under inflammatory conditions, they also modulated cytokines, chemokines, and neurotrophic factor release.

CONCLUSIONS: Yolkin and ovocystatin are non-toxic to HT29 and HBEC5i cells and actively influence gut and blood–brain barrier function by regulating permeability and secretory activity. These findings support their potential as nutraceuticals to preserve barrier integrity and brain homeostasis during immune challenges.

## S1P38. INVESTIGATING GENE-ENVIRONMENT INTERACTIONS IN TUBEROUS SCLEROSIS COMPLEX: THE ROLE OF IL-6 MEDIATED INFLAMMATORY RESPONSE IN NEURODEVELOPMENT

Shivani Dogra, Justyna Zmorzyńska

*Laboratory of Developmental Neurobiology, International Institute of Molecular Mechanisms and Machines, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Tuberous Sclerosis Complex (TSC) is a rare genetic disorder caused by mutations in TSC1 or TSC2, genes that encode for hamartin and tuberin respectively, normally suppress mTORC1 signaling. Loss of this inhibition leads to mTOR pathway hyperactivation, resulting in abnormal cell growth and a broad spectrum of clinical manifestations, including benign tumors, epilepsy, and TSC-associated neuropsychiatric disorders (TANDs). Neuropsychiatric disorders such as autism spectrum disorder (ASD), anxiety, and intellectual disability show substantial inter-individual variability, even among patients with similar mutations, suggesting that environmental factors contribute alongside genetic predisposition to neurodevelopmental outcomes. Inflammation during early development is a potential modifier of TSC phenotypes. Epidemiological studies have linked maternal infection and prenatal immune activation with an increased risk of ASD in offspring, indicating that immune signals may interact with underlying genetic vulnerabilities to alter brain development. Among inflammatory mediators, interleukin-6 (IL-6) is a pleiotropic cytokine produced by multiple immune cell types in response to infection or tissue damage. IL-6 can cross the blood–brain barrier and has been implicated in neuroinflammatory and neuropsychiatric conditions, including ASD and intellectual disability.

AIM(S): To investigate gene-environment interactions by microinjecting IL-6 into the bloodstream of TSC background zebrafish larvae.

METHOD(S): Breeding and Genotyping of TSC2 fish, Microinjections (2dpf, 3dpf), Behavioral test (Open field test), WMAS, imaging, and analysis.

RESULTS: My preliminary data shows that IL-6 microinjections into the duct of Cuvier increase anxiety-like behaviors and induce repetitive stereotypical swimming patterns that may reflect ASD in TSC background.

CONCLUSIONS: Early inflammatory signaling, with a focus on IL-6 microinjections into the duct of Cuvier, influences neural development and behavior in a TSC background.

FINANCIAL SUPPORT: Sonata bis: 2023/50/E/NZ3/00252.

## S1P39. RAC1 PATHWAY AS A THERAPEUTIC TARGET FOR RESCUING BRAIN CONNECTIVITY DISTURBANCES UNDERLYING ANXIETY IN TUBEROUS SCLEROSIS COMPLEX

Klaudia Wojtal, Tomasz Dulski, Olga Doszyń, Justyna Zmorzyńska

*Laboratory of Developmental Neurobiology, International Institute of Molecular Mechanisms and Machines, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Tuberous Sclerosis Complex (TSC) is a genetic neurodevelopmental disorder caused by lossoffunction mutations in TSC1 or TSC2, which hyperactivate the mTORC1 pathway. One of the features of TSC is the emergence of TSCassociated neuropsychiatric disorders (TANDs), among which anxiety and intellectual disability is prevalent.

AIM(S): We aim to uncover therapeutic targets within the Rac1 pathway to rescue disruptions in proper neurodevelopment and diminish anxiety-like behaviors in tsc2-deficient zebrafish.

METHOD(S): Using transparent, externally developing zebrafish, we can model neurodevelopmental abnormalities in vivo via plasmid electroporation and measuring GTP-bound RAC1. Lightsheet microscopy was used and samples were sent for RNA sequencing.

RESULTS: Our previous work demonstrated that tsc-2deficient zebrafish exhibit disrupted axon bundling, a reduced width of the anterior commissure and anxietylike behaviors. We found elevated Rac1 levels in mutant tsc2-deficient zebrafish which correlated with observed anxiety-like behaviour. Importantly, Rac1 in-

hibitors reversed anxiety-like behaviour, as well as aberrant connectivity between brain hemispheres. Rac1 is a crucial factor in axon elongation during development. Transcriptomic profiling of brain samples revealed that Rac1 inhibition restored expression of the majority of differentially expressed genes in the tsc2 mutants back to wild-type levels. KEGG pathway analysis identified significant enrichment in mTOR signaling and focal adhesion, while GO terms highlighted processes including axon guidance, axogenesis and neuronal projection. Moreover, the mRNA levels of Rac1 upstream activators dock3, dock4 and elmo2 were also increased in mutant brains.

CONCLUSIONS: Collectively, these findings highlight the role of the Rac1 pathway in TSC pathology and point towards cytoskeletal dysregulation and aberrant axon development as potential mechanisms underlying TSC-associated neuropsychiatric disorders.

FINANCIAL SUPPORT: This work was supported by the OPUS Grant No. 2020/37/B/NZ3/02345, Sonata Bis grant No. 2023/50/E/NZ3/00252, both from National Science Centre, Poland.

## S1P40. MODELING PARKINSON'S DISEASE BY BILATERAL ADMINISTRATION OF PREFORMED FIBRILS OF A-SYNUCLEIN TO THE BRAINSTEM

Anna Alwani, Piotr Chmielarz

*Department of Brain Biochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: According to Braak's hypothesis, Parkinson's disease (PD) pathology may originate in the lower brainstem and spread rostrally before affecting the substantia nigra, which could explain the presence of non-motor prodromal symptoms such as sleep disturbances, depression, and anxiety preceding classical motor deficits. Experimental models reflecting this early-stage progression are crucial for understanding the mechanisms underlying prodromal PD.

AIM(S): The aim of this study was to develop and characterize an experimental mouse model of early-stage Parkinson's disease by bilateral stereotaxic administration of preformed fibrils of α-synuclein into the brainstem.

METHOD(S): In this study, we modeled early PD-like pathology in C57BL/6J mice by bilateral stereotaxic injections of α-synuclein preformed fibrils into the dorsal motor nucleus of the vagus nerve (DMV). Animals were sacrificed at 1, 3, and 6 months post-injection to assess the temporal progression of α-synuclein pathology. Brain tissues were collected, sectioned, and processed for fluorescent immunohistochemistry using markers for α-synuclein aggregates (phosphoS129), Tph-positive serotonergic neurons, and TH-positive dopaminergic neurons.

RESULTS: Fluorescence staining revealed that, one month after injection, α-synuclein aggregates were confined to the dorsal motor nucleus of the vagus nerve. At three months, pathological inclusions began to spread rostrally toward the raphe nuclei. By six months post-injection, α-synuclein aggregates were clearly detected within the raphe nuclei, indicating progressive propagation of synucleinopathy along anatomically connected pathways.

CONCLUSIONS: These findings demonstrate that brainstem administration of preformedd fibrils of α-synuclein recapitulates key features of PD pathology progression consistent with Braak's staging hypothesis. This model may provide a valuable platform for investigating early, non-motor mechanisms of Parkinson's disease and for exploring therapeutic strategies targeting prodromal stages.

FINANCIAL SUPPORT: This research work was supported by the National Science Centre (Poland) under grant number: 2024/53/N/NZ4/01662 (Preludium 23).

## S1P41. DOPAMINE SIGNALING IN WAKE-PROMOTING CLOCK NEURONS REGULATES NEURONAL PLASTICITY AND LOCOMOTOR RHYTHMICITY

Aya Dridi[1,2], Milena Damulewicz[1]

[1] *Department of Cell Biology and Imaging, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

[2] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Dopaminergic neurons provide presynaptic input to the circadian clock network in Drosophila melanogaster, positioning dopamine (DA) to gate clock outputs that shape sleep architecture and locomotor rhythmicity. Because sleep fragmentation and circadian disruption are common non-motor symptoms of Parkinson's disease (PD), we tested how PD-like dopaminergic dysfunction perturbs clock output circuitry, focusing on PDF$^+$small ventrolateral neurons (sLNVs) that show day–night structural plasticity.

AIM(S): Determine whether reduced DA signaling disrupts(i)day–night remodeling of PDF⁺ sLNv dorsal termini and(ii)phase-specific locomotor and sleep outputs, and to identify DA receptor pathways in PDF neurons.

METHOD(S): We chronically reduced DA synthesis by GAL4–UAS knockdown of pale (ple; tyrosine hydroxylase) and quantified sLNv terminal complexity at ZT2 *vs.* ZT14 with locomotor and sleep behavior. To model progression, we compared adults at 7 *vs.* 30 days. To minimize developmental compensation, we induced acute adult DA depletion by feeding 3-iodo-L-tyrosine (3-IY). Dop1R1, Dop1R2, or Dop2R were silenced specifically in PDF neurons to probe receptor contributions.

RESULTS: Controls exhibited higher sLNv arbor complexity at ZT2 than ZT14. ple KD abolished this rhythm in terminal complexity. Behaviorally, ple KD reduced total locomotor output and blunted both the evening activity peak and the dawn response, while overall sleep time was largely preserved but became more fragmented during the light phase. These phenotypes strengthened with age. Acute adult DA depletion via 3-IY recapitulated the structural and behavioral signatures of chronic ple knockdown. Receptor inhibition in PDF neurons was used to delineate DA pathways linking dopaminergic input to sLNv remodeling and circadian behavior.

CONCLUSIONS: Across chronic, acute, and neuron-specific manipulations, dopaminergic signaling is required to maintain day–night structural plasticity of PDF⁺ sLNv dorsal termini and to support phase-specific circadian locomotor outputs. Our research provides a mechanistic circuit framework to study PD-linked sleep and circadian dysfunction.

FINANCIAL SUPPORT: This project has received funding from the European Union's HORIZON 2023 research and innovation programme under the Marie Skłodowska-Curie grant nr. 101169474.

## S1P42. EFFECTS OF A-SYNUCLEIN AGGREGATION IN THE MIDGUT ON BRAIN CONDITION – DROSOPHILA MELANOGASTER MODEL

Magdalena Siwarga, Milena Damulewicz

*Department of Cell Biology and Imaging, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Parkinsons Disease (PD) is one of the most prevalent neurodegenerative disorders. α-synuclein (α-syn) aggregation in form of Lewy Bodies (LB) is present in most PD patients, thus contituting a major hallmark of this disorder. The initial Lewy pathology has already been shown to be formed in the dorsal motor nucleus of the vagus (DMV) or olfactory bulb by neuropathological studies, which makes it plausible for LBs formed in gut nerve plexuses to propagate via vagus nerve to the brain.

AIM(S): The study aims to establish the link between gut-derived α-syn aggregates and brain pathology, considering α-syn spread mechanisms, oxidative stress response and behavioral manifestations.

METHOD(S): Locomotor activity study, gene expression analysis, climbing assay, survival study, smurf assay and whole brain imaging were performed on transgenic flies expressing PD-linked α-syn[A53T] tagged with BiFC-Venus under the control of the midgut expression (mex) promoter.

RESULTS: We found no significant signal in whole brain imaging, moreover mutated flies tested negative in the Smurf assay.

CONCLUSIONS: Our results suggest that sole expression of α-syn[A53T] in the midgut does not condition its spread to the brain. Moreover, the intestinal barrier resumes intact in the experimental group.

## S1P43. MODULATION OF A-SYNUCLEIN PATHOLOGY IN CELLULAR MODELS OF PARKINSON'S DISEASE

Zuzanna Wilk, Justyna Barut, Katarzyna Maziarz, Piotr Chmielarz, Grzegorz Kreiner

*Departament of Brain Biochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Parkinson's Disease is a neurodegenerative disorder affecting nearly 9 million people worldwide. It is characterized by the progressive loss of dopaminergic neurons in the SN/VTA region, leading to motor dysfunction. Aggregation and spreading of misfolded α-synuclein (α-syn) are considered key factors contributing to neuron loss. Mechanisms of α-syn aggregation and its possible clearance pathways are thus intensely investigated.

AIM(S): Our aim was to examine the role of disruption of the endolysosomal pathway (ELP) in pathological α-syn aggregation. Moreover, we aimed to investigate the effect of ghrelin receptor ligands on α-syn aggregation, putatively through modulation of ELP.

METHOD(S): We investigated the disruption of the ELP in an in vitro model of α-syn aggregation induced by α-syn preformed fibrils (PFF) in primary hippocampal neurons using chloroquine (CQ; 5 μM, 10 μM, 12.5 μM) or genetic ablation of lysosomal ion channel TMEM175. Ghrelin receptor agonists were investigated in primary dopaminergic neurons. MK-0677, anamorelin and ghrelin were tested in three concentrations: 3 nM, 10nM, 100 nM. pS129-α-syn-positive aggregates were visualized by immunofluorescence staining combined with either NeuN or tyrosine hydroxylase (TH) labeling, followed by automated imaging and quantification.

RESULTS: CQ dose-dependently increased formation of α-syn aggregates while TMEM175 ablation unexpectedly reduced α-syn accumulation threefold. Furthermore, we observed reduction of α-syn aggregates in neurons treated with MK-0677 and anamorelin.

CONCLUSIONS: The ELP appears to be an important player in modulation of α-syn pathology. However, its mechanism seems complex; ghrelin receptor agonists have potential to reduce α-syn aggregate load in dopaminergic cells.

FINANCIAL SUPPORT: This research was funded by the National Science Centre, Poland (grant no. 2019/35/D/NZ7/03200, Sonata 15).

## S1P44. NEUROPROTECTIVE ACTION OF TRAMIPROSATE IN A ROTENONE-BASED CELLULAR MODEL OF PARKINSON'S DISEASE

Ajay Modi[1,2], Sheetal Maria[1,2], Jiří Damborský[3,4,5], Martin Marek[3,4,5], Amit Khairnar[1,2,6]

[1] *Center for Translational Medicine, ICRC, St. Anne's University Hospital, Brno, Czech Republic*

[2] *Faculty of Medicine, Masaryk University, Brno, Czech Republic*

[3] *Loschmidt Laboratories, Faculty of Science, Masaryk University, Brno, Czech Republic*

[4] *RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic*

[5] *ICRC, St. Anne's University Hospital, Brno, Czech Republic*

[6] *Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*

INTRODUCTION: Parkinson's disease (PD) is a chronic neurodegenerative disorder marked by the progressive degeneration of dopaminergic neurons and pathological aggregation of α-synuclein (αSyn). Mitochondrial dysfunction, oxidative stress, and neuroinflammation are key contributors to disease progression. Rotenone, a mitochondrial complex I inhibitor, is commonly used to reproduce PD-like cellular pathology. Tramiprosate, an amyloid aggregation inhibitor developed for Alzheimer's disease, has recently shown potential neuroprotective effects; however, its role in PD has not yet been fully explored.

AIM(S): To investigate whether tramiprosate protects human dopaminergic SH-SY5Y cells from rotenone-induced neurotoxicity and to elucidate the underlying molecular mechanisms.

METHOD(S): SH-SY5Y cells were pre-treated with tramiprosate (1–400 μM) before rotenone exposure, and viability was evaluated using the Alamar Blue assay. Mechanistic studies were conducted using 20 μM tramiprosate pre-treatment for 24 h, followed by gene expression analysis (RT-PCR) and protein localization studies (immunocytochemistry).

RESULTS: Tramiprosate significantly enhanced cell survival across all tested concentrations. At 20 μM, tramiprosate restored tyrosine hydroxylase (TH) expression and reduced phosphorylated αSyn. It selectively altered αSyn isoform expression by increasing αSyn-140 and APOE levels while decreasing αSyn-126 and αSyn-98, with no change in αSyn-112. Tramiprosate also reduced oxidative stress–associated p47phox transcripts, without affecting NOX2 or NOX4. Inflammatory markers TNF-α and NFκB were downregulated, whereas the anti-inflammatory cytokine IL-10 was elevated. Furthermore, tramiprosate decreased both the number and size of lipid droplets despite an overall increase in total αSyn expression.

CONCLUSIONS: In conclusion, tramiprosate exerts strong neuroprotective effects in a rotenone-based cellular model of PD by modulating αSyn processing, oxidative stress, inflammation, and lipid metabolism.

FINANCIAL SUPPORT: We acknowledge the financial supports provided by the project "VGF peptides as protective agents against neurodegeneration" (MUNI/11/EDUC HE/2025); the European Union–funded project "Alzheimer's Disease Diagnostics Innovation and Translation to Clinical Practice in Central Europe" (Grant No. 101087124); and "Od preklinického ke klinickému výzkumu 2" (MUNI/A/1795/2025).

## S1P45. EVALUATION OF NEUROPROTECTIVE POTENTIAL OF HISTAMINE H3 RECEPTOR (H3R) ANTAGONISTS IN CELLULAR MODELS OF APOPTOSIS

Aleksandra Mąsior[1], Katarzyna Szczepańska[2], Danuta Jantas[1]

[1] *Department of Brain Biochemistry, Maj Institute of Pharmacology of the Polish Academy of Sciences, Kraków, Poland*

[2] *Department of Medicinal Chemistry, Maj Institute of Pharmacology of the Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Growing evidence suggests that histamine H3 receptor (H3R) antagonists exert neuroprotective effects, particularly under excitotoxic and oxidative stress conditions. As apoptosis drives neuronal loss in Parkinson's disease (PD), targeting these pathways is a promising therapeutic strategy.

AIM(S): Here, we examined selected H3R antagonists in cellular models of PD-relevant apoptosis to assess their potential to preserve neuronal survival.

METHOD(S): Four H3R antagonists: ciproxifan (CPX), JNJ-5207852 (JNJ), clobenpropit (CB) and pitolisant (Pit) at concentrations 0.01-10 uM were tested in human neuroblastoma SH-SY5Y cells exposed to pro-apoptotic agents: the dopaminergic neurotoxin $MPP^+$ and inducer of extracellular apoptosis, doxorubicin (DOX). Experiments were performed in undifferentiated (UN-) and retinoic acid (RA-) differentiated cells. Cytotoxicity (LDH release) assay was used for assessment of neuroprotection whereas caspase-3 activity was measured as a marker of apoptosis.

RESULTS: In the $MPP^+$ model only CPX (1–10 uM) exerted modest protective effects in UN-SH-SY5Y cells, whereas JNJ, CB, and Pit were ineffective. In RA-SH-SY5Y cells, none of the tested compounds attenuated the $MPP^+$-induced cytotoxicity. In both cell phenotypes, $MPP^+$-evoked increase in caspase-3 activity which was attenuated by Pit (10 uM) in UN-SH-SY5Y cells, and by CPX (10 uM), JNJ (1 uM) and Pit (10 uM) in RA-SH-SY5Y cells. In the DOX model of apoptosis neuroprotection demonstrated JNJ (0.1-10 uM), CB (0.01 and 1 uM) and Pit (0.01 and 1 uM) and this effect was observed only in UN-SH-SY5Y cells. Moreover, the Dox-evoked caspase-3 activity in UN-SH-SY5Y cells was significantly attenuated by CB (10 uM) and Pit (1 uM).

CONCLUSIONS: The H3R antagonists exhibit some neuroprotective potential in cellular models of apoptosis, however this effect is dependent on the type of pro-apoptotic inducer, type of H3R antagonist and cell differentiation status.

FINANCIAL SUPPORT: The study was supported by the statutory funds of Maj Institute of Pharmacology Polish Academy of Sciences.

## S1P46. DECODING PALMITOYLATION CHANGES IN PARKINSON'S DISEASE FIBROBLASTS

L. Murillo-Hernández[1,2,3], L. Muñoz-Delgado[2,3,4], P. Mir[2,3,4], A. Fontán-Lozano[1,2], R. Mejías[1,2]

[1] *Department of Physiology, Faculty of Biology, University of Seville, Seville, Spain*

[2] *Movement Disorders Unit, Neurology Service, Institute of Biomedicine of Seville, Virgen del Rocío University Hospital, University of Seville, Seville, Spain*

[3] *Network Center for Biomedical Research in Neurodegenerative Diseases, Carlos III Institute of Health, Madrid, Spain*

[4] *Department of Medicine, Faculty of Medicine, University of Seville, Seville, Spain*

INTRODUCTION: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the degeneration of the nigrostriatal pathway, which is essential for motor control. S-acylation, also known as protein palmitoylation (PP), is a reversible lipid post-translational modification (PTM) that regulates protein function. A previous pilot study revealed differences in the cortical palmitome of PD patients.

AIM(S): The aim of this study was to characterize the expression and PP levels in dermal fibroblasts from sporadic PD patients compared to controls to identify potential peripheral biomarkers.

METHOD(S): We examined the expression and PP levels of proteins in dermal fibroblasts from sporadic PD patients and controls (n=4-8 per group). Total and palmitoylated proteins were isolated using an acyl-biotin exchange assay (ABE), followed by western blotting and proteomic.

RESULTS: ABE-WB analyses of human fibroblasts suggested potential palmitoylation of the PD-related proteins. Notably, proteomic analyses revealed dysregulation of pathways implicated in PD pathophysiology. Palmitome analysis of the same samples identified 30 differentially palmitoylated proteins (DPPs) in PD patients compared to controls.

CONCLUSIONS: Our findings suggest distinct proteomic and palmitoylation signatures in dermal fibroblasts from sporadic PD patients, supporting the use of this cell model for detecting PD-associated PP alterations, investigating their pathological mechanisms, and facilitating the future development of biomarkers based on this PTM.

FINANCIAL SUPPORT: This work was supported by the Spanish Ministry of Science and Innovation (PID2023-150376OB-I00 – Knowledge Generation Projects 2023). Lucía Murillo is supported by a predoctoral research contract for training of research personnel from the Junta de Andalucía / CUII and the FSE+ (DGP_PRED_2024).

## S1P47. A NOVEL UNILATERAL RAT MODEL OF PARKINSON'S DISEASE COMBINING UBIQUITIN–PROTEASOME SYSTEM INHIBITION AND PATHOLOGICAL ALPHA-SYNUCLEIN AGGREGATION IN THE NIGROSTRIATAL PATHWAY

Klaudiusz Górka, Anna Alwani, Piotr Chmielarz, Adam Bielawski, Grzegorz Kreiner, Jolanta Konieczny

*Department of Brain Biochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Parkinson's disease (PD) is primarily characterized by clinical symptoms such as muscle rigidity, tremor and bradykinesia, resulting from the irreversible degeneration of dopaminergic (DA) neurons in the substantia nigra (SN). α-Synuclein aggregation in DA neurons plays a key role in PD pathogenesis, ultimately leading to neuronal death. Whilst animal models based on intracerebral administration of α-Synuclein fibrils into the nigrostriatal pathway faithfully reproduce early stages of PD, they do not induce DA neuron loss fast enough to generate robust motor deficits. In contrast, intranigral administration of the ubiquitin–proteasome system inhibitor lactacystin causes rapid dopaminergic neurodegeneration and motor symptoms, yet fails to reliably reproduce α -Synuclein aggregation typical of human PD.

AIM(S): The aim of this study was to develop a novel PD model combining intranigral administration of α-Synuclein fibrils with a low dose of lactacystin, thereby more closely resembling human PD pathology.

METHOD(S): Stereotactic unilateral administration of vehicle (2 µl PBS), lactacystin (0.5 µg), α-Synuclein fibrils (8 µg), or a combination of lactacystin and α-Synuclein fibrils into the SN was performed in male Wistar rats, which were subsequently subjected to a series of non-invasive behavioral tests assessing forelimb use asymmetry, including the cylinder test, forelimb stepping test, and vibrissae-evoked forelimb placing test.

RESULTS: Vehicle-, lactacystin-, and α-Synuclein fibril-treated rats showed no significant forelimb asymmetry at 2 and 4 weeks post-surgery, whilst in contrast, the combined administration of lactacystin and α-Synuclein fibrils exhibited a significant reduction in use of the compromised forelimb across all tests.

CONCLUSIONS: This novel model induces significant motor deficits indicative of severe nigrostriatal impairment, offering a promising and efficient platform for studying PD mechanisms and evaluating therapeutic strategies.

FINANCIAL SUPPORT: This work was supported by the statutory funds of the Department of Brain Biochemistry, Maj Institute of Pharmacology, PAS.

## S1P48. EFFECTS OF AN 8-WEEK AEROBIC INTERVAL TRAINING ON HAND MOTOR CONTROL AND CORTICAL ACTIVITY IN PARKINSON'S DISEASE

Karolina Stankowska, Karolina Lorek, Tomasz Ściepuro, Jarosław Marusiak

*Faculty of Physiotherapy, Wrocław University of Health and Sport Sciences, Wrocław, Poland*

INTRODUCTION: Previous studies suggest that intensive physical exercise may improve motor and cognitive symptoms in Parkinson's disease (PD); however, the underlying mechanisms remain unclear.

AIM(S): This study aimed to evaluate the effects of an 8-week aerobic interval training (AIT) program on clinical neurological outcomes and motor-related cortical potentials (MRCP-EEG) in patients with PD.

METHOD(S): Thirty-two patients with PD were assigned either to a training group (PD-TR, n=16), which completed an 8-week AIT program on a cycle ergometer, or to a non-trained group (PD-NTR, n=16), which continued standard treatment. Disease severity was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS) before (PRE) and after (POST) the intervention. EEG signals were recorded during a precision pinch task performed with the PD-affected limb. MRCP-EEG analysis focused on the early (BP1) and late (BP2) components of the readiness potential.

RESULTS: Following the AIT program, the PD-TR group showed significant improvements in UPDRS scores, including overall motor performance (sum of scores of UPDRS section III), manual dexterity (UPDRS 3.4 and 3.5), and overall functional status (total score of UPDRS sections I–IV). Behavioral improvements were accompanied by neurophysiological changes, specifi-

cally a significant increase in BP1 mean amplitude at the ipsilateral motor cortex. A session × group interaction and a significant difference in delta values (POST *vs.* PRE) were observed between PD-TR and PD-NTR groups.

CONCLUSIONS: An 8-week AIT program enhances motor performance in patients with PD, potentially through neurophysiological adaptations as evidenced by MRCP-EEG. These findings support the use of structured aerobic interval training as a complementary intervention for PD.

FINANCIAL SUPPORT: Wroclaw University of Health and Sport Sciences, Faculty of Physiotherapy, 51-612 Wrocław al. Ignacego Jana Paderewskiego 35.

## S1P49. IDENTIFICATION OF FACTORS STIMULATING CORNEAL WOUND HEALING

Anna Pacwa[1,2,3], Bartosz Machna[1,4,5], Klaudia Mróz[1,4], Anna Gąsiorek[1,4], Monika Katan[1,4], Maciej Osęka[5], Joanna Lewin-Kowalik[1,2,4], Adrian Smędowski[1,2,4,6,7]

[1] *The Laboratory for Translational Research in Ophthalmology, Department of Ophthalmology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

[2] *Glaucotech Co, Katowice, Poland*

[3] *Department of Clinical Genetics and Rare Diseases, Faculty of Medical Sciences in Katowice, Medical University of Silesia in Katowice, Katowice, Poland*

[4] *Department of Ophthalmology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

[5] *Oftalabs, Wałbrzych, Poland*

[6] *Department of Ophthalmology, Professor K. Gibinski University Clinical Center, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

[7] *Department of Paedriatric Ophthalmology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

INTRODUCTION: Corneal wound healing is a complex process involving epithelial cells recovery and tissue integrity restoration. Disruptions in this process can cause permanent damage and impaired vision. Currently, intensive research is being conducted on substances that support corneal regeneration.

AIM(S): The aim of this study was to determine a safe dose of D-Panthenol, Cabergoline and Pergolide to evaluate their effects on the wound healing process in cultures of human corneal epithelial cells (HCE-2).

METHOD(S): HCE cells were exposed to increasing concentrations of D-Panthenol (0.5-15%), Cabergoline (0.001-100 µM) and Pergolide (0.25-2 µg/mL). After 6, 12, 24 and 48 hours of incubation, a cell viability assay (Alamar Blue) and a cytotoxicity assay (RealTime-Glo) were performed. The positive control was an increasing concentration of H2O2 and the negative control was cells in culture medium. Based on the results, the optimal doses of the test substances were selected: D-Panthenol 1%, Cabergoline 100 µM, Pergolide 1 µg/mL to assess wound healing, which was carried out in Ibidi Culture-Insert 2 Well in µ-Dish 35 mm chambers. To assess cell proliferation and migration, microscopic images were taken of the cell culture exposed to the test substances at different time points.

RESULTS: Based on the tests performed, safe doses of the substances were established: D-Panthenol 1%, Cabergoline 100 µM and Pergolide 1 µg/mL. Analysis of microscopic images showed increased proliferation and migration of HCE cells compared to controls after exposure to Pergolide at a concentration of 1 µg/mL. The other supplements had no effect on HCE migration

CONCLUSIONS: The results showed that Pergolide may be a promising candidate for future regenerative therapies, especially in the context of treating corneal epithelial damage.

FINANCIAL SUPPORT: This project was conducted in collaboration with Oftalabs.

## S1P50. L-TYROSINE AS A DOPAMINE PRECURSOR: SYSTEMIC SUPPLEMENTATION SUPPORTS RETINAL FUNCTION IN A MOUSE MODEL OF FORM-DEPRIVATION MYOPIA

Bartosz Machna[1,2,3], Klaudia Mróz[1,2], Monika Katan[1,2], Anna Gąsiorek[1,2], Anna Pacwa[1,2,6,9], Maciej Osęka[3], Mikołaj Górka[5,7], Joanna Lewin-Kowalik[1,2,6], Adrian Smędowski[1,2,4,6,8]

[1] *Laboratory for Translational Research in Ophthalmology, Department of Ophthalmology, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

[2] *Department of Ophthalmology, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

[3] *Oftalabs Sp. z o.o., Wałbrzych, Poland*

[4] *Department of Ophthalmology, Professor K. Gibinski University Clinical Center, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

[5] *Department of Biochemistry and Medical Genetics, Faculty of Health Sciences, Medical University of Silesia, Katowice, Poland*

[6] *Glaucotech Co, Katowice, Poland*

[7] *Department of Experimental Medicine, Medical University of Silesia, Katowice, Poland*

[8] *Department of Paedriatric Ophthalmology, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

[9] *Department of Clinical Genetics and Rare Diseases, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

INTRODUCTION: High myopia is a growing global public health concern due to its rising prevalence and association with severe sight-threatening complications, including myopic maculopathy, retinal detachment, and glaucoma. Preclinical studies show reduced retinal dopamine signaling in form-deprivation myopia (FDM). As L-tyrosine is the precursor for dopamine synthesis, systemic supplementation is hypothesized to modulate ocular growth and retinal function in myopia.

AIM(S): This study aims to examine the effects of L-tyrosine supplementation on ocular morphometry and retinal function in a mouse model of form-deprivation myopia.

METHOD(S): Four-week-old C57BL/6 mice were used in a form-deprivation myopia model induced through unilateral tarsorrhaphy (eyelid suturing). Animals were randomly divided into two groups: an experimental group with a subcutaneous implantation of a slow-release L-tyrosine delivery system, and a control group subjected to a sham procedure. After the treatment period, axial length and corneal diameter at limbus were measured on high-resolution images using ImageJ software. Retinal function was evaluated by full-field electroretinography (ERG), including scotopic, mixed, photopic, and oscillatory potential responses.

RESULTS: Form-deprivation induced by tarsorrhaphy resulted in significantly greater ocular growth in eyes subjected to tarsorrhaphy (FDM). This growth was more pronounced in the sham-treated group compared to eyes of mice receiving systemic L-tyrosine. Electroretinographic recordings showed that tarsorrhaphy-treated eyes exhibited submaximal responses compared to the contralateral healthy eye. L-tyrosine application led to improved retinal function, particularly in rod responses, mixed responses, photopic negative responses, and oscillatory potentials.

CONCLUSIONS: L-tyrosine supplementation shows potential role in regulating emmetropization and preserving retinal bioelectrical responses. These results support further exploration of therapeutic potential in myopia management and prevention.

FINANCIAL SUPPORT: Ministry of Science and Higher Education (Poland), "Doktorat wdrożeniowy" programme, grant no. DWD/6/0407/2022.

## S1P51. LIGHT POLLUTION AS A DRIVER OF RETINAL AGEING AND NEURODEGENERATION IN DROSOPHILA MELANOGASTER

Kinga Skoczek[1,2], Milena Damulewicz[2]

[1] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

[2] *Department of Cell Biology and Imaging, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Retinal ageing is followed by visual decline and disturbed circadian regulation. Artificial light at night (ALAN) and blue light disrupt circadian rhythms and may promote neurodegeneration, however, their impact on retinal neurodegeneration and ageing still remains poorly characterized. Bruchpilot (BRP), a presynaptic protein forming active zones in photoreceptor terminals, enables efficient neurotransmission in the first visual neuropil of Drosophila. The α subunit of the $Na^+/K^+$-ATPase (αATP), present in epithelial glial cells, maintains ionic homeostasis, supporting synaptic transmission and circadian regulation. Both BRP and αATP are regulated by light and the circadian clock, showing a distinct pattern with peaks at the beginning of day and night, serving as markers of visual system clock alterations.

AIM(S): This study investigates the combined effect of aging and light pollution conditions on circadian rhythmicity in the visual system.

METHOD(S): Male CantonS flies were kept under standard light (LD12: 12), dim light at night (L-DIM), or 1h of blue light before night (LBD), aged to 7, 30 or 60 days. Flies were decapitated at four time points; heads were fixed,cryosectioned and immunostained for BRP or αATP. Immunofluorescence in the lamina was quantified by confocal microscopy. In addition, retinas were dissected at 6 time points, brp and αATP mRNA levels in young and old flies were measured by qPCR.

RESULTS: Under LD12: 12, young flies displayed rhythmic BRP and αATP expression in the lamina, which weakened with age. L-DIM and LBD caused additional shifts in the protein expression. qPCR showed that rhythms in retina differ from those in lamina and vary with age and exposure to light pollution.

CONCLUSIONS: Our results show that ageing weakens circadian regulation in the Drosophila visual system, indicated by molecular changes both in the photoreceptors and glial cells, with artificial light accelerating that process. These changes suggest that disrupted circadian control may contribute to retinal ageing.

FINANCIAL SUPPORT: 2022/47/B/NZ3/00250.

## S1P52. EFFECT OF LIGHT POLLUTION ON RETINAL AGEING IN THE DROSOPHILA MELANOGASTER MODEL

Monika Mitka[1,2], Kinga Skoczek[1,2], Milena Damulewicz[1]

[1] *Department of Cell Biology and Imaging, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

[2] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Artificial light at night (ALAN), a major component of light pollution, has emerged as a global environmental issue due to its disruptive effects on biological rhythms and physiological functions. Even short-term exposure to blue light emitted by device screens, especially just before bedtime, may dysregulate circadian machinery and affect sleep. The retina is the tissue most directly exposed to light, making it highly vulnerable to continuous artificial illumination. In Drosophila, the retina has an autonomous peripheral clock that participates in the regulation of activity and sleep and can serve as a model to study age- and light-related changes in circadian mechanisms.

AIM(S): Investigating the effects of aging and light pollution on the retinal peripheral clock in Drosophila.

METHOD(S): Wild-type flies were kept under standard light conditions LD 12:12 (12 h of light and 12 h of darkness), L-dim (12 h of light and 12 h of dimmed light), and LBD (12 h of light, 1 h of blue light, and 11 h of darkness). Young (7 days) and old (30 days) males were collected at specific time points of the day, their retinas were manually dissected, and clock gene expression was analyzed using qPCR. Sleep and locomotor activity assays were performed on young (2 days) and aged (30 days) flies kept under LD, L-dim, and LBD conditions. Finally, the walking optomotor response was assessed in young flies (7 days) under LD and LBD, and in old flies (30 days) under LD, LBD, and L-dim conditions.

RESULTS: Retinal ageing and different light conditions (L-dim and LBD) resulted in alterations in the daily pattern of clock gene expression. The rhythmicity of the optomotor walking response was lost in LBD and aged flies. Moreover, its accuracy was lower under L-dim conditions in aged flies. L-dim conditions also affected the pattern and quality of sleep in both young and aged flies.

CONCLUSIONS: Aging and light pollution affect the retinal peripheral clock. They also disrupt sleep patterns and quality, and impair visual motion processing.

FINANCIAL SUPPORT: NCN; 2022/47/B/NZ3/00250; Project leader: PhD Milena Damulewicz.

## S1P53. THE ROLE OF THE CIRCADIAN CLOCK IN THE DIGESTIVE SYSTEM IN THE REGULATION OF VITAL PROCESSES IN DROSOPHILA MELANOGASTER

Weronika Obrzut, Milena Damulewicz

*Department of Cell Biology and Imaging, Institute of Zoology and Biomedical Sciences, Jagiellonian University*

INTRODUCTION: Circadian rhythms are endogenous ~24-hour oscillations that regulate physiological processes and rhytmic regulation of gene expression. In Drosophila melanogaster, circadian organization is controlled by a central brain clock and peripheral clocks in many tissues, including the midgut. Rhythmic expression of core clock genes in the intestine suggests that the gut clock may influence metabolism, behavior, fitness, survival, and communication with the central nervous system.

AIM(S): This study investigated the role of the intestinal circadian clock in regulating physiological and behavioral processes in Drosophila melanogaster. We examined whether silencing the period (per) gene in

the midgut affects locomotor activity, survival, intestinal gene expression, and the function of central PDF neurons.

METHOD(S): Tissue-specific silencing of per was performed using the GAL4/UAS system (mex-GAL4 > UAS-perRNAi). Locomotor activity and survival were analyzed in progeny. Expression of core clock genes (per, tim) and intestine-associated genes (ninaD, itd) was measured by qPCR in isolated midguts. The potential impact on PDF neurons was also assessed.

RESULTS: Silencing per in the intestine disrupted rhythmic expression of clock-controlled and functional genes in the midgut. Such alterations influenced physiological performance, survival, and behavioral rhythms, potentially through modified signaling between the intestinal clock and central PDF neurons.

CONCLUSIONS: The intestinal circadian clock appears to function as an active component of the circadian network in Drosophila melanogaster. Disruption of per in the midgut may affect not only local gene regulation but also systemic physiology and behavioral rhythms. These findings highlight the importance of peripheral clocks in coordinating vital processes and their interaction with central circadian mechanisms.

## S1P54. NEUROPROTECTIVE EFFECTS OF ESCITALOPRAM IN THE RETINA: ROLE OF BRAIN-DERIVED NEUROTROPHIC FACTOR

Klaudia Mróz[1,3], Bartosz Machna[1,3,4], Anna Pacwa[1,2,5], Mikołaj Górka[7], Anna Gąsiorek[1,3], Monika Katan[1,3], Joanna Lewin-Kowalik[1,3,5], Adrian Smędowski[1,3,5,6,8]

*[1] Laboratory for Translational Research in Ophthalmology, Department of Opthalmology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

*[2] Department of Clinical Genetics and Rare Diseases, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

*[3] Department of Ophthalmology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

*[4] Oftalabs, Wałbrzych, Poland*

*[5] Glaucotech Co, Katowice, Poland*

*[6] Department of Ophthalmology, Professor K. Gibinski University Clinical Center, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

*[7] Center for Experimental Medicine, Medical University of Silesia, Katowice, Poland*

*[8] Department of Paedriatric Ophthalmology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

INTRODUCTION: Escitalopram, a selective serotonin reuptake inhibitor (SSRI), exhibits neuroprotective properties by reducing oxidative stress and excitotoxicity. It may act by increasing the expression of brain-derived neurotrophic factor (BDNF) or decreasing the conductivity of gap junctions composed of connexin 36 (Cx36).

AIM(S): The aim of this study was to evaluate the effect of Escitalopram on retinal BDNF expression and retinal neuron function.

METHOD(S): The study used wild-type mice and mice with partial bdnf knockout. The animals received oral Escitalopram (20 mg/ml) or PBS as a control every other day for three months. Retinal function was assessed using electroretinography (ERG), while BDNF expression was analyzed using Western blot and immunohistochemical staining techniques.

RESULTS: Western blot analysis showed lower BDNF expression in BDNF KO mice, whereas Escitalopram increased its levels in WT mice and partially compensated for the BDNF deficiency in KO mice. ERG analysis demonstrated that Escitalopram improved the function of the inner retinal layers, increasing the amplitudes of oscillatory potentials and the PhNR response, which may promote neuronal survival under ischemic conditions.

CONCLUSIONS: The neuroprotective effects of Escitalopram in the retina may be partially dependent on BDNF. SSRIs may regulate BDNF expression and improve retinal neuron function, suggesting their potential application in the treatment of ischemic retinal diseases.

## S1P55. IMPACT OF SELECTIVE SYNAPTIC TRANSMISSION BLOCKADE ON VISUAL SIGNAL CONDUCTION AND OXIDATIVE STRESS IN THE RAT RETINA

Monika Katan[1,2], Anna Pacwa[1,2,3,4], Anna Gąsiorek[1,2], Klaudia Bugara[1,2], Bartosz Machna[1,2], Joanna Lewin-Kowalik[1,2,3], Adrian Smędowski[1,2,3,5,6]

*[1] Laboratory for Translational Research in Ophthalmology, Department of Ophthalmology, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

*[2] Department of Ophthalmology, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

*[3] Glaucotech Co, Katowice, Poland*

*[4] Department of Clinical Genetics and Rare Diseases, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

*[5] Department of Ophthalmology, Professor K. Gibinski University Clinical Center, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

*[6] Department of Paediatric Ophthalmology, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

INTRODUCTION: Disturbances in retinal synaptic transmission may contribute to neurodegenerative processes and alter bioelectrical responses of the visual system.

AIM(S): This study investigated the effects of selective blockade of retinal synaptic transmission on visual evoked potentials (VEP) and oxidative stress markers in rats.

METHOD(S): The study included 40 Long Evans rats. VEP were recorded before and after intravitreal administration of pharmacological modulators targeting glutamatergic (AMPA, NMDA, mGluR6), GABAergic, and electrical synapses. VEP latency and amplitude were analyzed, and retinas were subsequently collected for immunofluorescence assessment of oxidative stress markers (4-HNE, 8-OHdG).

RESULTS: Selective synaptic modulation induced pathway-specific changes in VEP parameters. AMPA receptor blockade caused marked VEP latency prolongation (~31%) and amplitude reduction (~43%). NMDA receptor blockade resulted in a pronounced amplitude decrease (~55%) accompanied by latency shortening (~14%). GABAergic modulation led to strong amplitude reduction (~56%) and moderate latency prolongation (up to ~20%), whereas mGluR6 modulation produced milder effects. Retinal levels of the lipid peroxidation marker 4-HNE were significantly increased after AMPA, NMDA and GABAergic blockade ($p<0.0001$), indicating enhanced oxidative damage associated with disrupted synaptic transmission. In contrast, the DNA oxidation marker 8-OHdG was reduced after NMDA receptor blockade and GABAergic modulation ($p<0.0001$), suggesting predominance of early lipid-related oxidative responses.

CONCLUSIONS: Selective interference with retinal synaptic transmission leads to measurable functional alterations along the visual pathway accompanied by pathway-specific oxidative stress responses. The combined use of VEP recordings and oxidative stress markers provides a sensitive approach for assessing retinal synaptic dysfunction.

## S1P56. FROM GENES TO VISION LOSS: A COMPARATIVE STUDY OF RETINAL DYSTROPHY MOUSE MODELS

Anna Gąsiorek[1,2], Anna Pacwa[1,2,3,4], Monika Katan[1,2], Klaudia Mróz[1,2], Bartosz Machna[1,2], Xiaonan Liu[1,2,5], Joanna Lewin-Kowalik[1,2,3], Adrian Smędowski[1,2,3,6,7]

*[1] Laboratory for Translational Research in Ophthalmology, Department of Ophthalmology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

*[2] Department of Ophthalmology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

*[3] Glaucotech Co, Katowice, Poland*

*[4] Department of Clinical Genetics and Rare Diseases, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

*[5] Institute of Biotechnology, HiLIFE, University of Helsinki, Helsinki, Finland*

*[6] Department of Ophthalmology, Professor K. Gibinski University Clinical Center, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

*[7] Department of Paediatric Ophthalmology, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Katowice, Poland*

INTRODUCTION: The retina's excitable part consists of light-sensing photoreceptor cells that play a key role in phototransduction. Retinal dystrophies (RDs) are a group of degenerative disorders leading to photoreceptor damage and progressive vision loss. Regrettably, the majority of RDs are inherited and multiple gene defects have been identified with more than 270 genes, which make the diagnosis very challenging. It is noteworthy that over 25% of individuals with RDs carry mutations in yet unidentified genes, and the development of novel transgenic animal models offers new insight into their discovery.

AIM(S): The aim of this study was to characterize and compare transgenic mouse models of RDs.

METHOD(S): In this study, we used mutant mice (Rpe65, Abca4, Pde6b, Gucy2e, Cep290) that provide models for RDs, such as retinitis pigmentosa, Leber congenital amaurosis or Stargardt macular dystrophy. Retinal function and structure were assessed using electroretinography (ERG), Masson's trichrome staining, Western blot and immunofluorescence of selected proteins, apoptosis and oxidative stress markers.

RESULTS: Our findings show that, although all the mutants employed pertain to RDs, they exhibit distinct functional impairments in ERG and divergent phenotypic manifestations. ERG changes appeared in juvenile animals, with structural alterations evident in aged mice. Pde6b and Cep290 mutants showed complete photoreceptor loss with absence of the outer retinal layers. We also observed increased photoreceptor apoptosis, indicated by elevated Caspase-8 staining and higher levels of oxidative stress markers (HNE, 8-OHdG).

CONCLUSIONS: Although no universal cure exists, recent advances in gene therapy and stem cell transplantation show promise. Given the polygenic and heterogeneous nature of RDs, the continued development of advanced genetic animal models is vital for understanding disease mechanisms and creating targeted treatments.

## S1P57. NEURAL CORRELATES OF FAMILIAR FACE RECOGNITION: ARE FACES RECOGNIZED THROUGH A SERIES OF PROCESSING STAGES?

Milena Bojdo[1,2], Deni Zakriev[1], Maya Schipper[1], Maria Ciocan[1], Linda H. Lidborg[1], Holger Wiese[1]

*[1] Department of Psychology, Durham University, Durham, United Kingdom*

*[2] Centre for Brain Research, Jagiellonian University, Kraków, Poland*

INTRODUCTION: We are highly efficient at recognizing familiar people's faces. According to classic face recognition models, this process involves serial activation of (i) perceptual representations, which are then compared to (ii) domain-selective long-term face representations, enabling access to (iii) domain-general person (semantic, episodic, or affective) representations. However, recent research questioned this architecture, specifically the evidence for the separation of perceptual and post-perceptual processing stages.

AIM(S): This study aimed to re-examine the serial model using a newly developed experimental approach, investigating the impact of manipulating the activation of distinct face representations on event-related potential (ERP) responses elicited by highly personally familiar faces compared to unfamiliar faces.

METHOD(S): Experiment 1 manipulated contrast information in familiar and unfamiliar face images to disrupt their perceptual processing. Experiment 2 used cross-domain repetition priming, presenting identity-congruent or identity-incongruent names before familiar or unfamiliar faces to elicit the activation of domain-general person representations.

RESULTS: Experiment 1 found an early effect of contrast negation on the amplitude of the N170 component. In turn, the familiarity effect, with more negative amplitudes elicited by personally familiar relative to unfamiliar faces over occipito-temporal channels, emerged 200ms after stimulus onset. This effect was reduced in contrast-negative conditions. Experiment 2 detected the same familiarity effect between 200 and 300ms. Here, it was enhanced by the presentation of identity-congruent names before familiar faces only in the later 300-400ms time window.

CONCLUSIONS: Together, these findings provide nuanced and renewed support for the classic face recognition models, demonstrating separate processing stages that reflect the serial activation of perceptual (N170), long-term facial (200–300 ms), and domain-general (300–400 ms) representations.

FINANCIAL SUPPORT: The study was conducted as part of Durham University's Master's by Research program.

## S1P58. SEX DIFFERENCES IN LOW-GAMMA AUDITORY ENVELOPE FOLLOWING RESPONSES

Aurimas Mockevičius[1,2], Inga Griškova-Bulanova[1,2]

*[1] Vilnius University, Medical Science Center, Translational Health Research Institute, Vilnius, Lithuania*

*[2] Vilnius University, Life Sciences Center, Institute of Biosciences, Vilnius, Lithuania*

INTRODUCTION: Envelope-following responses (EFRs) provide a robust, noninvasive assessment of gamma-range cortical synchronization. While sex influences brain structure, auditory processing, and neuropsychiatric risk, sex-related variation in gamma-range auditory entrainment remains insufficiently characterized.

AIM(S): We aimed to test whether the ability to drive cortical networks in the low-gamma range differs between females and males using a click-based chirp-like auditory stimulus covering 30–60 Hz.

METHOD(S): Electroencephalography was recorded from 80 healthy young adults (42 females; mean age

26.07 years). To reduce hormonal-state variability, females were assessed during the early follicular phase. Time–frequency responses were quantified across nine fronto-central electrodes using phase-locking index (PLI) and event-related spectral perturbation (ERSP), and individual gamma frequency (IGF) was estimated as the frequency eliciting maximal synchronization or power.

RESULTS: Males exhibited significantly stronger responses than females, with higher PLI in the 35–43 Hz range and higher ERSP in the 35–46 Hz range (Bonferroni-corrected $p<0.05$). In contrast, IGFs did not differ between sexes and no hemispheric lateralization effects were observed.

CONCLUSIONS: These findings demonstrate sex-related differences in low-gamma auditory EFR strength but not in resonance frequency, underscoring the need for sex-specific normative baselines in clinical and translational applications of gamma-range auditory paradigms.

FINANCIAL SUPPORT: This study was supported by the Research Council of Lithuania (LMTLT agreement no. S-LJB-20-1).

## S1P59. BECOMING WHOLE: A NEUROEDUCATIONAL FRAMEWORK FOR COGNITIVE RESILIENCE ACROSS NESTED TIMESCALES

Anastasia Ajeng Wulan Tantri[1,2], Christiyanti Aprinastuti[3,4]

[1] *The Excellent Study, Learning Sciences, Kalasan, Yogyakarta, Indonesia*

[2] *Rewiring Hope, Center for Integrative Learning and Cognitive Resilience, Yogyakarta, Indonesia*

[3] *Faculty of Teacher Training, Universitas Sanata Dharma, Sleman, Yogyakarta, Indonesia*

[4] *Faculty of Mathematics, University of Debrecen, Debrecen, Hungary*

INTRODUCTION: Students experiencing emotional dysregulation, trauma, or chronic academic pressure often show reduced engagement, limited cognitive flexibility, and weakened learning motivation. Current educational interventions rarely address the neurophysiological mechanisms underlying these difficulties, particularly the rapid neural processes that interact with developmental and sociocultural factors.

AIM(S): This study aims to propose a neuroeducational framework for cognitive resilience across three nested timescales: (1) momenttomoment neural dynamics, (2) developmental trajectories, and (3) sociocultural influences. The goal is to link neurophysiological markers—especially eventrelated potentials (ERP)—to observable learning behaviors in adolescents.

METHOD(S): A conceptual analysis was conducted integrating evidence from affective neuroscience, developmental psychology, and educational research. ERP components related to visual attention, affective processing, and cognitive control were mapped onto behavioral patterns seen in Indonesian students experiencing emotional stress. Complementary physiological measures such as heartrate variability (HRV) and behavioral indicators were incorporated to illustrate a multimodal approach.

RESULTS: The analysis suggests that ERP markers can capture transient fluctuations in attention and emotion that correspond with classroom disengagement. Developmental factors—including emotional maturation and stress responsivity—moderate these neural responses, while sociocultural pressures amplify cognitive load. Resilience emerges as a dynamic interaction between neural stability, developmental adaptation, and environmental support.

CONCLUSIONS: The framework highlights how momentlevel neural processes shape longterm learning outcomes. It provides a foundation for designing neuroscienceinformed pedagogical strategies to support motivation, healing, and cognitive resilience in diverse student populations.

FINANCIAL SUPPORT: No external financial support was received for this work.

## S1P60. EEG PATTERNS IN BODY DYSMORPHIC DISORDER: A CLINICAL Q PROTOCOL

Seyed Mohammadreza Alavizadeh[1], Somaye Entezari[2]

[1] *Department of Sport Psychology, Tehran Institute of Clinical Sport Neuropsychology, Tehran, Iran*

[2] *Department of Clinical Psychology, Tehran Institute of Clinical Sport Neuropsychology, Tehran, Iran*

INTRODUCTION: Body Dysmorphic Disorder (BDD) is characterized by distressing preoccupations with perceived defects in appearance, often linked to abnormalities in visual-emotional processing and cognitive control. While quantitative EEG (QEEG) studies have explored neural correlates of BDD, rapid and clinically feasible assessment protocols remain underutilized.

AIM(S): This study investigates the utility of the ClinicalQ protocol (Swingle, 2015) a brief, 5-site EEG screening method, in identifying distinct electrophysiological patterns in individuals with BDD.

METHOD(S): 32 participants meeting DSM-5 criteria for BDD and 32 same age gender-matched healthy controls underwent a standardized ClinicalQ assessment. EEG data were recorded from five scalp locations (F3, F4, Fz, Cz, O1) during eyes-open, eyes-closed, and reading tasks. Absolute and relative power in delta (2 Hz), theta (3–7 Hz), alpha (8–12 Hz), SMR (13–15 Hz), beta (16–25 Hz), and high-beta/gamma (28–40 Hz) bands were computed. Group comparisons and correlation analyses with symptom severity scores were performed with BDD-YBOCS (Philips, et.al. 1997).

RESULTS: Preliminary analyses revealed that the BDD group exhibited significantly in elevated theta/beta ratio at F3/F4, reduced alpha power at O1, and increased high-beta activity at Fz compared to controls. These patterns were positively correlated with mention clinical measures, including preoccupation severity or anxiety scores.

CONCLUSIONS: The ClinicalQ protocol captures distinct EEG signatures associated with BDD, particularly in frontal and occipital regions implicated in emotional regulation and visual processing. These findings support the potential of rapid EEG screening as a complementary tool for early identification and neurofeedback treatment planning in BDD. Further research with larger samples is warranted to validate these markers.

FINANCIAL SUPPORT: Personal.

## S1P61. THINK TOUGH. ERP CORRELATES OF EMBODIED VERB MEANING PROCESSING

Szczepan Grzybowski, Magdalena Kwiatkowska, Paweł Kudela
*Institute of Applied Psychology, Jagiellonian University, Kraków, Poland*

INTRODUCTION: The theory of language embodiment posits that words' meanings are grounded in sensorimotor circuits in the brain. The cognitive processes involved in verbal material recognition would depend, then, on bodily actions and sensations. Embodied language processing was shown in neuroimaging (fMRI) studies for both the verb and noun classes of words.

AIM(S): We aimed to study the embodiment of the verb class based on the event-related potential of the brain. ERP technique, with its excellent temporal resolution and well-established correlates of semantic processing, can shed more light on the specifics of the processes involved in embodied words' meaning recognition.

METHOD(S): 50 right-handed, native Polish speakers viewed 80 verbs presented centrally for 800 ms each. 40 of the verbs denoted body part movements (20 arm-related actions, e.g., "to catch", and 20 leg-related actions, e.g., "to walk"), and 40 denoted abstract or "disembodied" concepts (e.g., "to think", "to desire"). All the stimuli used were matched on length and word frequency values. After each presentation, the participants had to decide whether they saw a concrete or an abstract word. Brain activity was measured with a 64-channel Biosemi Active Two EEG apparatus. Amplitude differences between the verb categories in the N400 and LPP time windows were analyzed. Behavioral data of post-stimulus categorization were also analyzed.

RESULTS: Higher N400 amplitudes were noted for concrete (movement or action-related) verbs as compared with the abstract ones, whereas higher LPP amplitudes were noted for the abstract verbs as compared with the concrete ones. Action verbs were also classified faster and more accurately.

CONCLUSIONS: Embodied, action-related verbs were shown to be processed differently by the brain. More resources were engaged during their semantic elaboration, and seemingly less effort was involved in the decision-programming stage. This was followed by an overall better behavioral performance for that verb category.

## S1P62. DEVELOPMENTAL TRAJECTORIES OF EEG SIGNAL COMPLEXITY AND BEHAVIORAL VARIABILITY FROM CHILDHOOD TO ADULTHOOD: A MULTISCALE ENTROPY APPROACH

Brenda Y Angulo-Ruíz, Vanesa Muñoz, Sheyla Andalia-Crespo, Elena I. Rodríguez-Martínez, Carlos M. Gómez
*Human Psychobiology Lab, University of Sevilla, Sevilla, Spain*

INTRODUCTION: The complexity of physiological signals provides important insight into the maturation and functional organization of the developing brain.

AIM(S): This study investigated age-related changes in electroencephalographic (EEG) signal complexity and their association with behavioural variability in a sample of 240 participants aged 6 to 29 years.

METHOD(S): Resting-state EEG was recorded, and Multiscale Entropy (MSE) was computed across 34 temporal scales grouped into fine, medium, and coarse

ranges. Behavioural variability was assessed using performance measures from Oddball and Delayed Match-to-Sample tasks. Quadratic regression analyses were conducted to characterize age-related changes in MSE across scalp regions, and Pearson correlations examined associations between age-adjusted MSE residuals and behavioural variability.

RESULTS: MSE showed significant age-related changes across temporal scales and cortical regions, with fine-scale MSE increasing across the scalp, region-specific decreases at medium scales, and a generalized decline at coarse scales. Behavioural variability decreased with age in both tasks. Notably, fine-scale MSE residuals in central and posterior regions were negatively correlated with the coefficient of variation in the Oddball task, indicating that higher neural complexity is associated with greater behavioural stability.

CONCLUSIONS: These findings demonstrate scale- and region-specific developmental trajectories of neural complexity and support the relevance of fine-scale MSE as a marker of brain maturation linked to behavioural stability beyond traditional variability measures.

FINANCIAL SUPPORT: This research was funded by Agencia Estatal de Investigación (AEI), grant number PID2022–139151OB-I00 (FEDER funds).

## S1P63. BEYOND LITERAL MEANING: BEHAVIORAL AND EEG EVENT-RELATED POTENTIAL CORRELATES OF FIGURATIVE LANGUAGE COMPREHENSION IMPAIRMENT IN SCHIZOPHRENIA – STUDY ON IDIOMS AND NOVEL METAPHORS

Ida Żyznowska[1], Wojciech Żukowski[1], Wiktor Więcławski[1], Suvarna Chinta[1], Piotr Błądziński[2,3], Aneta Kalisz[2,3], Andrzej Cechnicki[2,3], Krzysztof Walczewski[4,5], Łukasz Cichocki[4,5], Andrzej Marycz[5], Karolina Rataj[6], Jakub Szewczyk[1], Przemysław Adamczyk[1]

*[1] Institute of Psychology, Jagiellonian University, Kraków, Poland*

*[2] Department of Community Psychiatry, Association for the Development of Community Psychiatry and Care, Kraków, Poland*

*[3] Department of Biological and Community Psychiatry, Chair of Psychiatry and Psychotherapy, Jagiellonian University Medical College, Kraków, Poland*

*[4] Clinic of Psychiatry, Andrzej Frycz Modrzewski Kraków University, Kraków, Poland*

*[5] Babinski Clinical Hospital, Kraków, Poland*

*[6] Neuroscience of Language Laboratory, Department of Psycholinguistic Studies, Faculty of English, Adam Mickiewicz University, Poznań, Poland*

INTRODUCTION: Metaphors are crucial for social communication skills, which are reported to be impaired in schizophrenia.

AIM(S): This study investigates neural processing of two types of metaphor: idioms and novel metaphors in schizophrenia and healthy controls using behavioral and EEG-ERP methods.

METHOD(S): In behavior we tested 89 schizophrenia patients (SCH: 55M:34F, age 41±10.0) and 57 healthy controls (CON: 38M:19F, age 41±9.1). EGG results were reported from 50 SCH (35M, 15F; age: 43±9.3) and 42 CON (27M, 15F; age: 42±8.3). Participants performed two punchline-based 1) comprehension and 2) reading tasks. The stimuli comprised 120 short stories in four conditions: idioms (IDI), novel metaphors (NOV), literal (LIT), and meaningless (ABS). To identify between-group differences, we analyzed behavioral scores and two ERP components: N400 and P600. A 64-channel EEG was recorded and preprocessed for ERP analysis using MathWorks MATLAB Automagic. Linear mixed-effects models were fitted for each channel in the 300–500 ms (N400) and 500–700 ms (P600) windows. Group differences in N400 sensitivity to cloze probability (CP) were tested via the group × CP interaction and simple slopes analyses.

RESULTS: SCH showed significantly lower accuracy than controls for LIT ($p<0.01$) toward a trend for IDI ($p=0.06$), with no group differences for NOV and ABS items. In EEG, SCH exhibited altered N400 responses to LIT and ABS stories distributed fronto-centrally and to IDI and NOV at posterior regions. P600 differences for IDI and NOV were observed centro-parietally. CP slopes showed reduced sensitivity to word predictability in SCH distributed at frontal and centro-parietal sites.

CONCLUSIONS: To conclude, altered N400/P600 reflect poorer detection of metaphors in schizophrenia. The lower sensitivity to CP indicates less efficient contextual integration of language. We may assume that schizophrenia patients mainly struggle with conventional meanings and misinterpret literal stories as figurative, possibly as a compensatory strategy.

FINANCIAL SUPPORT: This study was supported by the National Science Centre Poland grant no. 2021/41/B/HS6/02967.

## S1P64. IMPACT OF BLINK ARTIFACT REMOVAL METHODS ON DIRECTED TRANSFER FUNCTION ESTIMATION IN EEG

Weronika Bakun[1,2], Joanna Duda-Goławska[2,3], Maciej Kamiński[1], Jarosław Żygierewicz[1]

[1] *Faculty of Physics, Biomedical Physics Division, University of Warsaw, Warszawa, Poland*

[2] *Faculty of Psychology, Cognitive Psychology and Neurocognitive Science Division, University of Warsaw, Warszawa, Poland*

[3] *Neurocognitive Development Lab, Institute of Psychology, Polish Academy of Sciences, Warszawa, Poland*

INTRODUCTION: Directed Transfer Function (DTF) is a measure, used in EEG analysis, that allows the identification of causal relationships between signals in the frequency domain. It is derived from the concept of Granger causality and is based on the analysis of a multivariate autoregressive (MVAR) model fitted to the data.

AIM(S): It is crucial to use the optimal preprocessing method before calculating DTF. In particular, Independent Component Analysis (ICA), the most commonly used method for removing ocular artifacts, reconstructs EEG signals as linear combinations of independent sources. While effective for artifact suppression, such linear recomposition may alter dependency patterns relevant for DTF estimation. The present study investigates how blink-removal methods affect DTF outcomes using a controlled experimental paradigm.

METHOD(S): Participants were instructed to blink in response to auditory cues across three 15-second blocks: (1) blinking every second, (2) blinking every five seconds, and (3) no blinking. Each block was repeated ten times. Four signal conditions were analysed: (i) original no-blink signal (assumed ground truth), (ii) blink-contaminated signal without correction, (iii) blink-contaminated signal cleaned using ICA, and (iv) blink-contaminated signal cleaned using analytical modelling of the artifact. DTF matrices were computed for each condition using identical MVAR modeling hyperparameters.

RESULTS: We hypothesise that ICA-based correction may introduce artificial dependencies detectable in DTF due to its linear-mixing framework, whereas parametric modelling – being temporally localised and non-mixing – may better preserve original information flow structure.

CONCLUSIONS: Preprocessing choices are not neutral with respect to effective connectivity analysis. For studies aiming to interpret DTF in terms of neurophysiological information flow, artifact-removal methods that minimize global signal recomposition should be preferred.

FINANCIAL SUPPORT: This work was supported by the European Union under the Horizon-Widera Europe program (grant agreement No. 101159414).

## S1P68. ARE YOU KIDDING ME? NEURAL CORRELATES OF IMPAIRED HUMOR PROCESSING IN SCHIZOPHRENIA – A BEHAVIORAL AND EEG-ERP STUDY

Przemysław Adamczyk[1], Ida Żyznowska[1], Wojciech Żukowski[1], Wiktor Więcławski[1], Suvarna Chinta[1], Piotr Błądziński[2,3], Aneta Kalisz[2,3], Andrzej Cechnicki[2,3], Krzysztof Walczewski[4,5], Łukasz Cichocki[4,5], Andrzej Marycz[5], Karolina Rataj[6], Jakub Szewczyk[1]

[1] *Institute of Psychology, Jagiellonian University, Kraków, Poland*

[2] *Department of Community Psychiatry, Association for the Development of Community Psychiatry and Care, Kraków, Poland*

[3] *Department of Biological and Community Psychiatry, Chair of Psychiatry and Psychotherapy, Jagiellonian University Medical College, Kraków, Poland*

[4] *Clinic of Psychiatry, Andrzej Frycz Modrzewski Kraków University, Kraków, Poland*

[5] *Babinski Clinical Hospital, Kraków, Poland*

[6] *Neuroscience of Language Laboratory, Department of Psycholinguistic Studies, Faculty of English, Adam Mickiewicz University, Poznań, Poland*

INTRODUCTION: Individuals with schizophrenia commonly exhibit impairments in the pragmatic use of humor, yet neural evidence remains scarce.

AIM(S): To address this gap, we aimed to differentiate EEG-ERP brain responses to two types of humor: intended-to-be-funny (kids) and social norm–violating (adults) jokes in individuals with schizophrenia and healthy controls.

METHOD(S): The behavioral study included 89 patients with schizophrenia (55M, 34F; age: 41±10.0) and 57 healthy controls (38M, 19F; age: 41±9.1), who rated the comprehensibility and figurativeness of 120 stories in four conditions: intended-to-be-funny (IFJ), social norm–violating (SVJ), and their literal (LIT) and nonsensical (ABS) control counterparts in a punchline-based humor comprehension task. EEG results are reported for 50 patients (35M, 15F; age: 43±9.3) and 42 controls (27M, 15F; age: 42±8.3). Participants read another 120 stories in a punchline-based humor reading task. A 64-channel EEG was recorded and preprocessed for ERP analysis using MathWorks MATLAB Automagic. Linear mixed-effects models were computed for

each channel in the 300–500 ms (N400) and 500–700 ms (P600) windows.

RESULTS: Behavioral results showed lower scores for IFJ, SVJ, and LIT, but not for ABS, in the clinical group ($p<0.002$). In EEG, N400 differences at parieto-occipital sites were found for SVJ and IFJ. At P600, the groups differed across all conditions over centro-parietal and parieto-occipital regions, with additional fronto-central differentiation for ABS and SVJ.

CONCLUSIONS: In conclusion, our results provide novel insight into the neural underpinnings of humor-processing impairment in schizophrenia. The diminished N400 suggests a reduced efficiency in incongruity detection and further attenuation of its resolution at P600. Together with behavioral results, our findings indicate less effective context integration processing in schizophrenia. In summary, this study offers a unique characterization of humor processing in neurotypical and clinical populations.

FINANCIAL SUPPORT: This study was supported by the National Science Centre Poland grant no. 2021/41/B/HS6/02967.

## S1P68. NEURAL MARKERS OF SARCASM AND IRONY PROCESSING IMPAIRMENT IN SCHIZOPHRENIA: A BEHAVIORAL AND EEG-ERP STUDY

Wojciech Żukowski[1], Ida Żyznowska[1], Wiktor Więcławski[1], Suvarna Chinta[1], Piotr Błądziński[2,3], Aneta Kalisz[2,3], Andrzej Cechnicki[2,3], Krzysztof Walczewski[4,5], Łukasz Cichocki[4,5], Andrzej Marycz[5], Karolina Rataj[6], Jakub Szewczyk[1], Przemysław Adamczyk[1]

[1] *Institute of Psychology, Jagiellonian University, Kraków, Poland*

[2] *Department of Community Psychiatry, Association for the Development of Community Psychiatry and Care, Kraków, Poland*

[3] *Department of Biological and Community Psychiatry, Chair of Psychiatry and Psychotherapy, Jagiellonian University Medical College, Kraków, Poland*

[4] *Clinic of Psychiatry, Andrzej Frycz Modrzewski Kraków University, Kraków, Poland*

[5] *Babinski Clinical Hospital, Kraków, Poland*

[6] *Neuroscience of Language Laboratory Department of Psycholinguistic Studies Faculty of English Adam Mickiewicz University, Poznań, Poland*

INTRODUCTION: Schizophrenia is a complex psychobiological disorder, in which neurodevelopmental changes lead to psychosocial and communication impairments, as e.g. pragmatic use of irony and sarcasm. Recent data on irony and sarcasm processing in schizophrenia is very limited.

AIM(S): Yet our study attempts to provide deeper insight into the neural correlates of this phenomenon using behavioral and EEG-ERP methods.

METHOD(S): In behavior, we tested 89 schizophrenia patients (55M: 34F, age 41±10.0) and 57 healthy controls (38M: 19F, age 41±9.1). Participants rated comprehensibility and figurativeness of 120 stories in four conditions: irony (IRO), sarcasm (SAR), and literal (LIT) and absurd (meaningless ABS). In EEG we reported results from 50 patients (35M, 15F; age: 43±9.3) and 42 controls (27M, 15F; age: 42±8.3). who read another 120 stories in the same four conditions. A 64-channel EEG was recorded and preprocessed for ERP analysis using MathWorks MATLAB Automagic. Linear mixed-effects models were computed for each channel in the 300–500 ms (N400) and 500–700 ms (P600) windows.

RESULTS: Behavioral results showed that patients were less accurate than controls in identifying SAR, IRO, and LIT, but not ABS ($p<.05$). In EEG, N400 between-group differences were found for SAR on parietal, parieto-occipital, and occipital regions. P600 differences emerged for ABS over frontal and central sites, and for both IRO and SAR over centro-parietal, parietal, and occipito-parietal regions. Notably, patients exhibited a general suppression of neural activity across all conditions and almost all electrodes.

CONCLUSIONS: In conclusion, this study is the first to demonstrate EEG-ERP correlates of irony and sarcasm processing in schizophrenia. Altered N400 and P600 responses indicate disrupted detection and integration of non-literal meaning, pointing to neural mechanisms underlying pragmatic communication impairments. Our study offers unique and valuable insight into impaired pragmatic language processing in schizophrenia.

FINANCIAL SUPPORT: This study was supported by the National Science Centre Poland grant no. 2021/41/B/HS6/02967.

## S1P69. EVOLUTIONARY THREAT SUPERIORITY? ENHANCED EVEN-RELATED POTENTIALS IN RESPONSE TO THREATENING ANIMALS COMPARED TO EMOTIONAL FACES

Pouria Motaghi[1], Rob van Lier[2], Rob van der Lubbe[3,4], Marijtje L. A. Jongsma[1]

[1] *Behavioural Science Institute, Radboud University, Nijmegen, The Netherlands*

[2] *Donders Institute for Brain, Cognition, and Behaviour, Radboud University, Nijmegen, The Netherlands*

[3] *Laboratory of Vision Sciences and Optometry, Faculty of Physics and Astronomy, Adam Mickiewicz University, Poznań, Poland*

[4] *Faculty of Behavior, Management and Social Sciences, University of Twente, Enschede, The Netherlands*

INTRODUCTION: Threat processing occurs rapidly, with responses emerging as early as 45 ms. EEG studies show that threat modulates event-related potential (ERP) components such as P1, N170, and EPN, reflecting early attention and emotional processing. Emotional facial expressions and threatening animals are widely used to study fear processing. However, a direct comparison between these two types of stimuli at the within-participant level has received little attention in the literature.

AIM(S): This study directly compares pictures of negative facial expressions and threatening animal pictures to evaluate and compare the threat response reflected on the ERPs between these two stimulus sets to determine which stimuli are most suitable to study threat processing.

METHOD(S): Forty healthy adults completed an EEG experiment involving passive viewing of faces and animals. Participants viewed 200 face pictures (angry, fearful, neutral, and inverted neutral) and 200 animal pictures (spider, snake, bird, and inverted bird), followed by a scrambled version of the animal pictures to control for the low-level visual features. A 64-channel EEG setup was used for the extraction of ERP components from four latency windows (P120, N170, P200, and EPN). We used repeated-measures ANOVA to assess the threat response by analyzing mean ERP amplitudes for each component.

RESULTS: ERP analyses revealed robust threat effect for animal stimuli across all components, with snake and spider pictures eliciting more negative amplitudes than birds, particularly over parietal and parieto-occipital regions. In contrast, facial expressions showed limited threat effects, with small differences emerging mainly in later components and specific regions of interest (ROIs).

CONCLUSIONS: Our findings show that threatening animal pictures evoke a stronger and earlier response to threat than emotional facial expressions. These results suggest that animal stimuli may provide a stronger and more reliable tool for studying rapid threat processing in EEG studies.

FINANCIAL SUPPORT: This research has been conducted within a self-funded PhD project.

## S1P70. NEURAL MARKERS OF THERAPEUTIC CHANGE IN COGNITIVE-BEHAVIORAL THERAPY FOR PROCRASTINATION: A RANDOMIZED CONTROLLED EEG TRIAL

Weronika Maria Browarczyk[1], Ewa Wiwatowska[1], Magdalena Pietruch[2], Joachim Kowalski[3], Marek Wypych[2], Jarosław M. Michałowski[1]

[1] *Poznań Laboratory of Affective Neuroscience, Institute of Psychology, SWPS University, Warsaw, Poland*

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

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

INTRODUCTION: Procrastination involves the voluntary delay of intended actions despite anticipated negative consequences. This prevalent self-regulatory difficulty is linked to impaired academic functioning and psychological distress. Although Cognitive-Behavioral Therapy (CBT) is an effective treatment, little is known about the neural mechanisms supporting therapeutic change. Longitudinal neurophysiological studies examining how CBT affects attentional and emotional processing in procrastination are scarce.

AIM(S): Using EEG in a randomized controlled design, this study investigates neurophysiological correlates of CBT for procrastination, focusing on treatment-induced changes in attentional bias and emotional processing of negative and procrastination-related lexical stimuli.

METHOD(S): Help-seeking university students with elevated academic procrastination were assigned to a brief, group-based CBT intervention or a wait-list control. EEG data were collected before and after the intervention while participants viewed procrastination-related, negative, and neutral single-word stimuli. Our analyses focus on ERP components indexing early attentional allocation and later stages of emotional and semantic processing, including the EPN, LPP, and N400.

RESULTS: Data collection from 135 participants is complete. The intervention effectively reduced self-reported procrastination, measured by the Aitken Pro-

crastination Inventory-Revised, compared to the control group. Neurophysiological analyses are currently underway.

CONCLUSIONS: We hypothesize that CBT will elicit measurable changes in emotion-related neurophysiological markers of attention. Specifically, we expect reduced early and late event-related responses to emotional stimuli, reflecting improved regulation of negative and procrastination-specific processing following the intervention. By identifying the neural processes underlying CBT, this study aims to provide a mechanistic understanding of therapeutic change and inform the development of more targeted interventions.

FINANCIAL SUPPORT: Supported by the National Science Centre, Poland, grant No. 2021/43/B/HS6/02024.

## S1P71. SHARED NEURAL AND BEHAVIORAL DYNAMICS OF MENTAL ROTATION ACROSS SEXES AND WOMEN'S HORMONAL STATUS GROUPS

Rimantė Gaižauskaitė, Ingrida Zelionkaitė, Ramunė Grikšienė

*Department of Neurobiology and Biophysics, Institute of Biosciences, Life Science Center, Vilnius University, Vilnius, Lithuania*

INTRODUCTION: Spatial abilities are essential for everyday functioning. The mental rotation task (MRT) has consistently shown one of the largest sex differences, typically favoring men. Some studies suggest that women's MRT performance may vary across the menstrual cycle or with hormonal contraception use, however, findings remain inconsistent. Sex differences in MRT performance may partly reflect variation in strategy used, highlighting the need to integrate behavioral and neural evidence to understand underlying mechanisms.

AIM(S): This study examined mental rotation performance and its neural correlates while considering sex differences and women's hormonal status.

METHOD(S): A total of 167 participants (35 men; 132 women across menstrual-cycle phases and contraceptive groups) completed a computerized MRT in which stimuli were presented at varying angular disparities (from 15° to 155°) while EEG was recorded. Accuracy and response times (RT) were analyzed alongside neural variables (rotation-related negativity (RRN) and global field power (GFP)). Salivary sex steroid concentrations were measured.

RESULTS: Mental rotation typically shows a gradual increase in RT and GFP, accompanied by decreasing RRN amplitude as angular disparity increases. Instead, both behavioral and neural measures showed nonlinear patterns: RT and GFP increased until ~75° before plateauing, whereas RRN amplitude decreased until ~55° and then remained constant. These patterns were consistent across sexes and hormonal groups and likely reflect transition from rotation to alternative strategies. Hormonal associations were minimal; however, testosterone showed a positive relationship with RRN amplitude in users of contraceptives with androgenic properties.

CONCLUSIONS: Together, these findings suggest that increasing angular disparity between figures in MRT evoke sex and women hormonal statuses independent changes in neural activity followed by adjustment in performance.

FINANCIAL SUPPORT: No external funding was received for this study.

## S1P72. NEURAL CORRELATES OF FAMILIARITY: FN400 MODULATION DURING ENCODING OF FOOD STIMULI

Delilah (Paulina) Wąsowska-Dean, Aneta Brzezicka

*Department of Psychology, Center for Neurocognitive Research, Warsaw, Poland*

INTRODUCTION: Recognition memory is often explained within dual-process models distinguishing familiarity and recollection. The FN400 event-related potential component has been associated with familiarity, yet its functional interpretation remains debated and is typically examined during retrieval rather than encoding. This interpretation remains debated, particularly regarding its relation to conceptual fluency or semantic priming.

AIM(S): This study investigated whether familiarity-related neural activity indexed by the FN400 can be observed during the encoding phase of a recognition paradigm, and whether habitual dietary inflammation status modulates this effect while using food as potentially ecologically valid stimuli.

METHOD(S): Middle-aged to older adults (M=57.3 years) completed an Old/New learning task during EEG recording with healthy, unhealthy, and control object stimuli. Participants were divided into pro-

and anti-inflammatory groups based on the Dietary Inflammatory Index (DII) calculated from Food Frequency Questionnaire responses. FN400 amplitudes (300-500) were analyzed using repeated-measures ANOVAs (including: Stimuli, Electrodes/Clusters, and Group).

RESULTS: No significant main effects of stimulus type, dietary group, or their interaction were observed for FN400 amplitudes (all p>.05), effect sizes were small. Significant findings were limited to topographic differences across electrode clusters and Electrode x Stimulus interaction. Exploratory inspection suggested possible early P2 modulation distinguishing food from control stimuli, indicating potential attentional or perceptual salience.

CONCLUSIONS: Findings suggest that FN400 familiarity effects may be less robust during encoding than retrieval. Methodological factors such as static food stimuli and categorical dietary grouping may have reduced sensitivity. Future research should employ continuous dietary measures, multisensory or personalised food stimuli, and broader ERP analyses to clarify familiarity-related neural dynamics during encoding.

## S2P01. A NOVEL CONTINUOUS SCORING APPROACH FOR LARGE-SCALE PHARMACOGENOMIC PROFILING IN THE UK BIOBANK

Jacek Hajto, Małgorzata Borczyk, Marcin Piechota, Sylwia Grubarek, Paula Konowalska, Dżesika Hoinkis, Michał Korostyński

*Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Pharmacogenomics (PGx) is moving toward a personalized approach, emphasizing the role of genetic individuality in optimizing pharmacotherapy. Biomedical datasets, such as the UK Biobank, provide opportunities to perform comprehensive genomic analyses and overcome the limitations of traditional pharmacogenetic classifications.

AIM(S): The study aims to conduct a comprehensive, large-scale pharmacogenomic investigation using data from 140,000 UK Biobank participants to explore the genetic determinants of drug response and find novel variants associated with clinical outcomes.

METHOD(S): We extracted clinical phenotypes from approximately 28 million primary care prescriptions and hospital admission records. We also integrated diverse genetic data types: ~1,5 million single-nucleotide polymorphisms, copy number variation, loss-of-function, and missense variants. For key pharmacogenes, we compared standard star-allele nomenclature against our custom tool, PharmGScore, which provides continuous functional scoring. Association analyses were conducted utilizing four statistical tools: PLINK2, LDAK, REGENIE, and CI-GWAS.

RESULTS: Our first results for warfarin dosing replicated established pharmacogenes (CYP2C9, VKORC1, CYP4F2), showing consistency with known determinants. Notably, continuous PharmGScore profiling of the CYP2C9 gene was highly significant (p<1e-43). Furthermore, utilizing PharmGScore to capture functional variations, we identified a significant association between CYP2C19 genetic burden and fluoxetine prescription (p=8.13e-5).

CONCLUSIONS: The UK Biobank is an exceptional database for large-scale medical data research and pharmacogenetic studies. Integrating deep longitudinal records with functional scoring allows for the discovery of novel variants and genes potentially linked to adverse drug reactions or specific drug treatments, paving the way for improved personalized therapy in somatic and psychiatric diseases.

FINANCIAL SUPPORT: Funding for this study was provided by National Science Centre, Poland PRELUDIUM BIS-3 nr 2021/43/O/NZ7/01187 and the Medical Research Agency with funds from the National Recovery and Resilience Plan (KPO) from the Recovery and Resilience Facility (RRF) KPOD.07.07-IW.07-0099/24.

## S2P02. ASSOCIATIONS OF GLUCOCORTICOID RECEPTOR–DEPENDENT GENE SIGNATURES WITH NEUROPSYCHIATRIC PHENOTYPES

Mateusz Zięba[1], Magdalena Ziemiańska[2], Małgorzata Borczyk[1], Marcin Piechota[1], Michał Korostyński[1]

[1] *Laboratory of Pharmacogenomics, Department of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Department of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Stress induces the hypothalamic–pituitary–adrenal (HPA) axis, leading to glucocorticoid release. These hormones signal through the glucocorticoid receptor (GR), which regulates genes involved in metabolism, immunity, and brain function. Prolonged HPA activity disrupts GR signaling, a hallmark of stress-related psychiatric disorders. However, GR-dependent gene expression regulation remains insufficiently characterized in the context of mental health.

AIM(S): Our study aims to evaluate the genetic overlap between systemic and tissue-specific GR-dependent signatures and genes previously associated with mental health disorders.

METHOD(S): We compiled a GR-dependent gene database from 47 publications, including 13,207 protein-coding genes. From this resource, we derived systemic and tissue-specific GR signatures across neural, blood, and lung tissues, stratified by direction of regulation. The signatures were tested for genetic overlap with mental health–associated gene sets from DisGeNET, GWAS Catalog, and GeneBass. Phenotypes were mapped to ICD-10 codes from mental and behavioural disorders. We analyzed 239 phenotypes (≥10 genes) using $\chi^2$ gene-overlap tests; significance was defined as $p<0.05$ and ≥3 overlapping genes.

RESULTS: We identified 388 GR-dependent genes significantly associated with 51 mental health phenotypes, most frequently linked to mood (affective) disorders (n=23). Notably, the direction of GR regulation differed between tissues: in the lung, associations were driven mainly by GR-repressed genes (p=0.02), whereas in neural tissue, they were stronger for GR-activated genes (p=0.04).

CONCLUSIONS: Together, these results indicate distinct central and peripheral roles of GR signaling in mental health. Neural associations are primarily driven by GR-activated genes enriched in synaptic and stress-response pathways, whereas lung associations involve GR-repressed genes linked to cytokine regulation.

FINANCIAL SUPPORT: This work was funded by NCN, Poland OPUS 23 2022/45/B/NZ5/03188.

## S2P03. HIGH-THROUGHPUT BEHAVIOR ANALYSIS REVEALS SEX-SPECIFIC CONTRIBUTION OF ASTROCYTE-DERIVED FACTORS TO EFFECTS OF CHRONIC CORTICOSTERONE AND KETAMINE IN MICE

Anna M. Lech[1], Patrycja Ziuzia[1,2], Bartosz Zglinicki[1], Martyna Skuła[1,3], Klaudia Kuzdrowska[1], Michał Ślęzak[1]

*[1] P4Health, Lukasiewicz Research Network – PORT Polish Center for Technology Development, Wrocław, Poland*

*[2] Department of Biochemistry and Molecular Biology, Wrocław University of Environmental and Life Sciences, Wrocław, Poland*

*[3] Department of Artificial Intelligence, Wrocław University of Science and Technology, Wrocław, Poland*

INTRODUCTION: Impaired synaptic homeostasis is a well-established feature of pathophysiology of psychiatric conditions. These deficits may originate in neurons, but glial cells are also known to mediate multiple aspects of synapse formation and elimination. Our earlier transcriptional profiling revealed crucial role of glucocorticoid receptor-dependent gene network in shaping the molecular profile of astrocytes in human depression and mouse chronic stress. One class of genes were astrocyte-specific synaptogenic factors. However, the contribution of specific proteins to plasticity of adult neural circuits and their potential as therapeutic targets remain unexplored.

AIM(S): The present work investigates how targeted manipulation of selected astrocyte proteins controlling synapse number shapes behavioral consequences of chronic stress and the response to antidepressant treatment.

METHOD(S): Gene silencing in medial prefrontal cortex was achieved through bilateral delivery of shRNA vectors containing a GFP reporter. Behavioral assessment of freely moving mice captured both, individual parameters – such as locomotion and speed movement, and social behaviors. A chronic corticosterone paradigm (CORT, 21 days in drinking water) was used to permanently activate the GR signaling. We quantified behavioral parameters in males and females across three time-points: baseline, after completion of CORT treatment, and 24h following a single subanesthetic dose of ketamine.

RESULTS: Immunostaining of GFP and astrocyte-specific markers confirmed the localization and the efficacy of gene knockdown. Principal Component Analysis (PCA) revealed that distinct behavioral components contributed to sex-specific effects of chronic CORT and ketamine.

CONCLUSIONS: These findings underscore the importance of astrocyte-derived synaptic regulators in glucocorticoid-dependent responses and advance our understanding of how glia shapes neural circuits in depression.

FINANCIAL SUPPORT: This research was funded by National Science Center grant number OPUS2021/41/B/NZ3/04099.

## S2P04. MODULATORY EFFECTS OF KETAMINE ON CORTICOSTERONE-DRIVEN SPINE ALTERATIONS IN PRIMARY HIPPOCAMPAL NEURONS

Krzysztof Kączek, Agnieszka Zelek-Molik, Michał Wilczkowski

*Department of Brain Biochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Dendritic spines are highly plastic structures that determine the strength and efficacy of excitatory synaptic transmission. Chronic elevation of glucocorticoids, such as corticosterone (CORT), disrupts spine morphology and contributes to synaptic dysfunction associated with stress-related disorders. In contrast, ketamine, a rapid-acting antidepressant, has been shown to promote synaptic remodeling and enhance structural plasticity in hippocampal circuits.

AIM(S): Therefore, we investigated whether ketamine reverses morphological alterations induced by chronic CORT exposure in primary mouse hippocampal cultures.

METHOD(S): Primary hippocampal neurons were exposed to CORT (250 nM) for 72 hours to induce stress-related spine remodeling. To assess the potential restorative effects of ketamine, the drug was applied during the final 24 hours of CORT incubation. Spine morphology was subsequently analyzed to evaluate changes in spine length, head width and spine density as these parameters are closely associated with efficacy and maturation of the synapse.

RESULTS: Ketamine treatment showed a tendency to reverse CORT-induced morphological changes. Ketamine restored also spine head dimensions, spine length and density toward control-like values.

CONCLUSIONS: These findings evidenced that ketamine exerts modulatory effects on stress-induced dendritic spine remodeling in hippocampal neurons and may partially counteract glucocorticoid-driven structural impairments. Our results support the hypothesis that ketamine's rapid antidepressant activity involves mechanisms associated with the restoration of synaptic structural plasticity.

FINANCIAL SUPPORT: Supported by the Polish National Science Center, No. 2021/41/N/NZ4/01845.

## S2P05. CHRONIC CORTICOSTERONE DIFFERENTIALLY ALTERS DENDRITIC SPINE MORPHOLOGY IN PRIMARY CORTICAL AND HIPPOCAMPAL NEURONS

Michał Wilczkowski, Anna Alwani, Agnieszka Zelek-Molik, Krzysztof Kączek, Irena Nalepa

*Department of Brain Biochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: The majority of excitatory chemical synapses in the brain are formed on dendritic spines, and even subtle alterations in dendritic spine morphology may impair neuronal network function. Therefore, identifying the biological factors that regulate dendritic spine structure is of major importance. One such factor is chronically elevated corticosterone (CORT). Chronic CORT has been associated with reduced dendritic spine number and structural alterations in cortical and hippocampal neurons.

AIM(S): However, it remains unclear whether CORT directly drives these morphological changes and, if so, to what extent and which specific structural parameters are altered by prolonged CORT elevation.

METHOD(S): To address this, we employed a simplified in vitro approach using mouse primary cells isolated from the cortex and hippocampus, and exposed primary cells to CORT for 72 h.

RESULTS: First, we experimentally identified a CORT concentration sufficient to induce a glucocorticoid signaling response without compromising primary cell viability or overall neuronal morphology. Next, we showed that prolonged CORT exposure differentially affects dendritic spine morphology in primary hippocampal and cortical neurons. In cortical neurons, we observed an increase in spine length, whereas in hippocampal neurons, mean spine length was reduced. In addition, primary cells from both regions exhibited decreased spine head width. Finally, we examined whether these CORT-induced structural alterations in dendritic spines are dependent on glucocorticoid receptor (GR) signaling by using CORT108297, a GR-selective antagonist.

CONCLUSIONS: Prolonged CORT exposure directly alters dendritic spine morphology in primary mouse neurons in a region-specific manner. Together, these findings suggest that CORT may directly affect dendritic spines and provide a detailed characterization of the associated structural alterations.

FINANCIAL SUPPORT: Supported by the Polish National Science Center, No. 2021/41/N/NZ4/01845.

## S2P06. EFFECTS OF PHARMACOLOGICALLY INDUCED CHRONIC AND ACUTE STRESS ON RAT BEHAVIOR IN THE OPEN-FIELD TEST: TOWARD A NOVEL RODENT MODEL OF PTSD

Klaudia Pszczółkowska[1,2], Bartosz Caban[1], Renata Bocian[1], Tomasz Kowalczyk[1]

[1] *Department of Neurobiology, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland*

[2] *Doctoral School of Exact and Natural Sciences, University of Łódź, Łódź, Poland*

INTRODUCTION: Stress is a common physiological response involving complex interactions between neurobiological and endocrinological factors. Numerous studies show that stress has a major contribution to the pathogenesis of multiple central nervous system dysfunctions, including post-traumatic disorder (PTSD), major depression disorder, or anxiety disorders. To provide better insight into the mechanisms underlying these diseases, rodent-based models are widely used to study the stress response. In this study, we introduce a pharmacologically induced stress model to assess PTSD-related behavioral markers using the open field test in parallel with wireless EEG recordings from the posterior hypothalamic area (PHa).

AIM(S): This study investigates the effects of corticosterone-induced chronic stress and combined chronic–acute stress on behavioral markers in a rodent model of PTSD.

METHOD(S): Sixteen male Wistar rats were divided into experimental (n=8) and control (n=8) groups. Rats from the experimental group were receiving constant doses of corticosterone for 21 days and were exposed to the open field test (OF) nine times. During OF testing, behavioral activity and bioelectrical signals from PHa were recorded continuously. Here, we report a subset of the behavioral analysis.

RESULTS: Behavioral analysis focused on established rodent PTSD markers, including locomotor activity, immobility, rearing, sniffing, grooming, and time spent in the center of the arena. Rats exposed to chronic and combined chronic–acute stress exhibited a pronounced increase in automatic behaviors, including grooming, fur licking, and sniffing. Behavioral differences observed between the experimental and control groups are discussed.

CONCLUSIONS: The combined model of monitoring both behavioral and bioelectrical activity in stressful conditions could be a possible tool in searching for the etiology and pharmacology of behaviors considered diagnostic criteria for PTSD, defined in the Diagnostic and Statistical Manual of Mental Disorders.

## S2P07. COMPARATIVE ANALYSIS OF DIFFERENT METHODS FOR SCORING SOCIAL CONTACT SEEKING IN ADOLESCENT MICE

Natalia Kurek[1], Klaudia Misiołek[1], Zofia Harda[1], Marta Klimczak[1], Magdalena Chrószcz[1], Łukasz Szumiec[1], Maria Kaczmarczyk-Jarosz[2], Aleksandra Rzeszut[1], Rafał Ryguła[3], Barbara Ziółkowska[1], Jan Rodriguez Parkitna[1]

[1] *Department of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Department of Physiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[3] *Affective Cognitive Neuroscience Laboratory, Department of Pharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: We observed that during mid-adolescence, mice show reduced sensitivity to social reward. Importantly, this effect is not attributable to decreased social investigation or to generalized deficit in reward processing. Quantification of social interactions is complex and susceptible to confounding factors. Human observers may introduce subjective bias, while automated tracking software carries the risk of over- or underfitting the behaviour classification criteria.

AIM(S): Our aim was to establish whether automatic analysis of social contact seeking produces results comparable to those obtained through manual scoring.

METHOD(S): Social contact seeking was assessed in a custom experimental cage (48 x 12 cm) that allowed interaction between two mice separated by a transparent, perforated divider. In automatic analysis using EthoVision, a social interaction was defined as a moment when the center point of the detected subject mouse (black pixel cluster) was present in a predefined zone adjacent to the divider. In manual scoring (BORIS), social investigation was defined as the active pursuit of social contact, with precisely defined onset and offset of the behaviour.

RESULTS: Both methods revealed no age-related differences in total social investigation time, indicating that automated scoring reliably detected behavioural patterns. However, automated analysis registered higher absolute values across all age groups. The results of the two methods were positively correlated, indicating that despite quantitative differences, the overall behavioural pattern was consistent across scoring approaches.

CONCLUSIONS: Human scoring of social behaviour remains the more precise and ethologically relevant

approach. However, automated analysis produces results relatively consistent with manual scoring, and resolves the issue of blinded scoring, and is significantly more time-efficient.

FINANCIAL SUPPORT: This work was supported by the Polish National Science Centre (grant number 2016/21/B/NZ4/00198).

## S2P08. THE ROLE OF LIPID-ASSOCIATED CIRCULATING MIRNAS IN THE INTERGENERATIONAL TRANSMISSION OF THE EFFECTS OF PARENTAL ADVERSE CHILDHOOD EXPERIENCES

Magdalena Gomółka[1,3], Weronika Tomaszewska[1,3], Sandra Binias[2], Regina Nadalinska[1], Ali Jawaid[1]

*[1] Translational Neuropsychiatry Research Group (TREND Lab), Life Sciences & Biotechnology Center Łukasiewicz Research Network PORT – Polish Centre of Technology Development, Wrocław, Poland*

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

*[3] Nencki Institute of Experimental Biology PAS, Warsaw, Poland*

INTRODUCTION: Adverse childhood experiences (ACE) have lasting negative effects on adult physical and mental health, and growing evidence suggests that ACE-related behavioral and metabolic alterations can be transmitted across generations.

AIM(S): This study investigates the role of lipid-associated circulating microRNAs (miRNAs) in mediating ACE effects to the germline for intergenerational transmission of behavioral and metabolic perturbations.

METHOD(S): ACE was modelled in mice using the well-established unpredictable maternal separation and stress (MSUS) paradigm with parallel modeling of lipid-modifying interventions; high fat diet (HFD) and voluntary exercise (VE). Intergenerational behavioral and metabolic phenotyping was performed across all cohorts of mice along with unbiased characterization of serum and sperm miRNAs through small RNA sequencing and RT-qPCR assays.

RESULTS: Both MSUS and HFD were associated with robust intergenerational behavioral and metabolic effects, including impaired glucose tolerance, and increased despair and anxiety-like behaviors in the offspring. Cross-injection studies confirmed the contribution of lipid-associated circulating miRNAs in germline embedding and intergenerational transmission of phenotypes. Specifically, injecting lipid-associated miRNA carriers from MSUS into control male mice recapitulated anxiety-like phenotypes, as well as impaired glucose tolerance in their offspring. On the contrary, cross-injections of lipid-associated miRNA carriers from VE mice into MSUS partially mitigated the metabolic perturbations associated with MSUS.

CONCLUSIONS: Together, these results provide 'proof-of-concept' evidence for the contribution of lipid-associated circulating miRNAs in the intergenerational transmission of ACE-related phenotypes. Ongoing studies are using conditional knock-out approaches in mice to further define the causal role of lipid-associated miRNAs in the intergenerational transmission of ACE effects.

FINANCIAL SUPPORT: National Science Centre (NCN) Poland; ERA Net Co-fund 2.

## S2P09. REGION-SPECIFIC EFFECTS OF CLONAZEPAM ON STEROIDOGENIC ENZYME GENE EXPRESSION IN THE RAT BRAIN

Karolina Kopeć, Katarzyna Bogus, Antonello Pinna, Aleksandra Suszka-Świtek, Artur Pałasz

*Department of Histology, Faculty of Medical Sciences, Medical University of Silesia, Katowice, Poland*

INTRODUCTION: Neurosteroids are synthesized de novo in the brain from cholesterol. This process resembles steroidogenesis in the gonads, adrenal glands, and placenta and involves P450scc, 3β-HSD, 17β-HSD, and aromatase. Dysregulated neurosteroid levels are linked to several neuropsychiatric disorders. Major brain regions involved in neurosteroid synthesis include the neocortex, striatum, hypothalamus, thalamus, hippocampus, amygdala, brainstem, and cerebellum. Benzodiazepines, by crossing the blood–brain barrier, can directly affect these brain structures.

AIM(S): To evaluate the effect of clonazepam on neurosteroidogenesis in the rat brain.

METHOD(S): Two-month-old male SPD rats were housed at 22°C under a 12/12-h light/dark cycle with free access to food and water. Animals received NaCl (control) or clonazepam (0.5 mg/kg, i.p.) for 21 days. After decapitation, eight brain regions were isolated. RNA was extracted with TRIZOL, quality assessed spectrophotometrically, and reverse-transcribed to cDNA. Expression of P450scc, 3β-HSD, 17β-HSD, aromatase, and B2M was quantified by qRT-PCR using SYBR Green.

RESULTS: Clonazepam increased 3β-HSD gene expression in the amygdala and p450scc mRNA in the hippocampus. Increased 3β-HSD is associated with enhanced allopregnanolone synthesis, whereas elevated hippocampal p450scc suggests increased pregnenolone levels. Clonazepam decreased P450scc protein levels in the neocortex, striatum, amygdala, thalamus, hypothalamus, cerebellum, and brainstem, and reduced 3β-HSD in the neocortex, striatum, hippocampus, thalamus, hypothalamus, cerebellum, and brainstem. It also inhibited 17β-HSD and aromatase gene expression in all regions.

CONCLUSIONS: Clonazepam modulates neurosteroidogenesis in a region-specific manner, providing insight into its pharmacological mechanisms and supporting further investigation of benzodiazepine effects on brain steroid pathways.

FINANCIAL SUPPORT: This research was funded by the Medical University of Silesia grant for the Department of Histology No; BNW-1-005/N/4/I.

## S2P10. EFFECTS OF MULTIMODAL COMPOUND HBK-15 ON CA1 CYTOARCHITECTURE IN A MOUSE MODEL OF EXCITATORY HYPOFUNCTION

Paulina Dudzik[1,2], Jorge Valero[3,4,5], Carla Escudero-Solano[3,4,5], Luís Antonia de Castro[3,4], Angelika Jagielska[1,2], David Pérez-Boyero[3,4], Jimena Pérez-Arévalo[3,4], Concepción Lillo[3,4], Klaudia Lustyk[1], Karolina Pytka[1]

*[1] Laboratory of Experimental Neuropharmacology, Faculty of Pharmacy, Chair of Pharmacodynamics, Jagiellonian University Medical College, Kraków, Poland*

*[2] Doctoral School of Medical and Health Sciences, Jagiellonian University Medical College, Kraków, Poland*

*[3] University of Salamanca, Salamanca, Spain*

*[4] INCYL, Institute of Neuroscience of Castilla y León, Salamanca, Spain*

*[5] IBSAL, Institute of Biomedical Research of Salamanca, Salamanca, Spain*

INTRODUCTION: Hippocampal excitatory hypofunction has been implicated in cognitive deficits across multiple neuropsychiatric disorders. Excitatory impairments may alter the microglial state, contributing to persistent circuit dysfunction. Multimodal compounds, such as HBK-15, represent a potential strategy for addressing this complex pathophysiology by simultaneously engaging various targets that regulate neurotransmission. HBK-15 exhibits high affinity for the 5-HT1A, 5-HT7, and sigma-1 receptors.

AIM(S): Accordingly, this study aimed to assess the effects of HBK-15 on hippocampal microglia in a mouse model of MK-801-induced excitatory impairment.

METHOD(S): During the reversal phase of cognitive flexibility assessment, for eight days, adult male mice were treated daily with HBK-15 (0.625, 1.25, and 2.5 mg/kg), followed by MK-801 (0.15 mg/kg) administration. Microglial changes in the CA1 region of the hippocampus were evaluated using immunohistochemistry for Iba1. Volumetric estimations of the CA1 region were performed via Cavalieri's method.

RESULTS: Exploratory analysis indicated that MK-801 administration was associated with a modest decrease in Iba1 immunoreactivity in the CA1 region compared to saline-treated controls, as reflected by a reduced area fraction covered by Iba1+ microglia. Treatment with HBK-15 appeared to normalize these deficits. Volumetric analysis showed no detectable effect of MK-801 on CA1 volume, however, HBK-15 at the lowest dose increased total CA1 volume compared with the control and MK-801 groups.

CONCLUSIONS: Preliminary findings suggest that HBK-15 modulates the structural organization in the CA1 region of the hippocampus, while its potential role in microglial responses requires further investigation.

FINANCIAL SUPPORT: Co-funded by the European Union under Erasmus+ Cooperation Partnership programme (2023-1-PL01-KA220-HED-000160284). Science and Innovation Spanish Ministry (PID2022-140525NB-I00 and PID2022-140456NB-I00). Strategic plans and strategic research programmes of excellence from the Regional Government of Castile and León, co-funded by the ERDF Operational Programme (CLU-2023-1-01).

## S2P12. METABOLISM, REDOX BALANCE AND BEHAVIOUR OF TRAP1 MUTANT MICE – A NEW MITOCHONDRIA-LINKED MODEL OF ASD

Dorota Malicka[1], Monika Puchalska[2], Katarzyna Radwańska[2], Magdalena Dziembowska[1], Bożena Kuzniewska[1]

[1] *Laboratory of Molecular Neurobiology, Faculty of Biology, University of Warsaw, Warsaw, Poland*

[2] *Laboratory of Molecular Basis of Behavior, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Neuronal cells critically depend on mitochondrial activity to maintain membrane excitability, neurotransmission, and synaptic plasticity. Accordingly, mitochondrial dysfunction has been observed in autism spectrum disorders (ASD), although the causal relationships remain unclear.

AIM(S): In an individual with ASD whose identical twin was unaffected, we identified a mutation (p.Q639*) in the TRAP1 gene. TRAP1 is a mitochondrial chaperone belonging to the HSP90 family and plays a key role in regulating cellular metabolism and protecting against oxidative stress. To investigate the functional consequences of TRAP1 loss in vivo, we generated knock-in Trap1 p.Q641* mice. These mice display social behavior deficits, which are more pronounced in males.

METHOD(S): We next performed a comprehensive behavioral assessment of Trap1 mutant mice of both sexes. To investigate whether TRAP1 deficiency affects brain metabolism, we performed mass spectrometry–based proteomic analyses of two brain regions frequently affected in ASD, the prefrontal cortex and hippocampus.

RESULTS: Across a series of behavioural tests, mutant mice did not manifest overt phenotypic disparities compared to wild-type controls, with the exception of variations in acclimatisation to novel environments and previously documented social deficiencies. These analyses revealed sex-dependent effects of the Trap1 mutation on protein expression profiles, pointing to alterations in metabolic pathways in the brain. Given the central role of mitochondria in metabolic regulation, we further assessed redox balance, a key indicator of mitochondrial function and metabolic state.

CONCLUSIONS: Together, these data indicate that TRAP1 deficiency leads to metabolic alterations in the prefrontal cortex and hippocampus in a sex-specific manner in this genetic mouse model of ASD.

FINANCIAL SUPPORT: This work was supported by the National Science Centre, Poland, under research project OPUS 2023/51/B/NZ4/00856.

## S2P13. THE ROLE OF SPERM MICRORNAS IN THE INTERGENERATIONAL TRANSMISSION OF THE EFFECTS OF ADVERSE CHILDHOOD EXPERIENCES

Agata Małoburska[1], Weronika Tomaszewska[1], Regina Nadalinska[1], Magdalena Gomółka[1], Sandra Binias[2], Taufik Hidayat[1], Ali Jawaid[1]

[1] *Translational Neuropsychiatry Research Group, Łukasiewicz Research Network – PORT Polish Center for Technology Development, Wrocław, Poland*

[2] *Laboratory of Sequencing, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Adverse childhood experiences (ACE) are critical determinants of adult physical and mental health in humans. Emerging evidence from rodent studies further supports that their effects extend to subsequent generations. In particular, patrilineal transmission of neuropsychiatric and metabolic vulnerability has been linked to ACE-induced changes in sperm non-coding RNAs, especially microRNAs.

AIM(S): We aim to understand the translational relevance of this novel concept, as well as the signaling cascades mediating germline miRNA changes after ACE.

METHOD(S): We systematically examined small RNAs in the sperm samples from 36-42 years old Bosnian men (n=22, n=20 controls), who were directly exposed to the Srebrenica genocide during childhood using unbiased small RNA sequencing (sRNA-seq) followed by RT-qPCRs. In addition, comprehensive neuropsychiatric assessments were performed in the fathers and their children, and the symptoms were correlated with the paternal sperm miRNAs to evaluate the biological plausibility of intergenerational transmission.

RESULTS: sRNA-seq identified differential expression of 5 sperm miRNAs between the ACE and the control groups. RT-qPCR assays further confirmed decrease of miR-26b-5p and trend towards decrease of miR-29c-3p in the sperm of genocide-exposed individuals compared to controls. Importantly, levels of these miRNAs were significantly correlated with depression, anxiety, and attention deficit hyperactivity disorder (ADHD) – related symptoms in the children.

CONCLUSIONS: Collectively, these findings underscore the potential role of sperm miRNAs in the intergenerational transmission of ACE-related phenotypes in humans and support the candidacy of certain miRNAs as biomarkers and potential mediators of such effects.

## S2P14. NONPARAMETRIC CIRCADIAN RHYTHM ESTIMATION USING EXTENDED RAIN

Piotr Biegański, Monika Tutaj
*Faculty of Physics, Biomedical Physics Division, University of Warsaw, Warszawa, Poland*

INTRODUCTION: RAIN is a nonparametric method developed to detect circadian activity in data which underlying period is not well-approximated by a sine. While widely used in research on gene expression, its formulation makes its use limited to sparsely sampled data, e.g. once every hour. At the same time, a method capable of detecting non-sinusoidal rhythmicity and assigning statistical significance to the given period would be a valuable tool in different fields connected to chronobiology, like actigraphy—which stands for measurement of movement intensity—due to asymmetrical nature of human sleep/wake cycle.

AIM(S): To develop a method for nonparametric estimation of asymmetric periodicity.

METHOD(S): We present an extension of RAIN, which changes the way it groups datapoints and the way it handles multiday recordings by aggregation of points in windows and estimating net p-value for a multiday recording, while preserving probability distribution underlying the zeroth hypothesis in the original estimator. Such reformulation is carried to make the algorithm usable on data sampled with higher sampling rates.

RESULTS: The benchmarks on synthetic data exhibit correct detection of underlying rhythmicity, while allowing use of higher sampling rates. In comparison with the cosinor method (which stands for fitting a sine using the least squares optimization), the presented version of RAIN exhibits better detection of asymmetric oscillations in data.

CONCLUSIONS: Presented rhythm detection methodology is a step forward in comparison with original RAIN, allowing nonparametric detection of asymmetric periods in biological data with higher sampling rates, however it needs further development in order to make it more computationally efficient and robust to rhythmicity changes across days during a multiday recording.

## S2P15. ORIGIN AND LOCALIZATION OF IGFBP2 IN REGULATION OF DENDRITIC SPINE STRUCTURAL PLASTICITY

Magdalena Rutkowska, Diana Legutko, Piotr Michaluk, Leszek Kaczmarek
*Nencki Institute of Experimental Biology PAS, Department of Neurobiology, Warsaw, Poland*

INTRODUCTION: Insulin-like growth factor 1 (IGF1) and its receptor (IGF1R) constitute as a crucial regulator in dendritic spine structural plasticity. However, extracellular IGF1 is mainly bound to its binding protein 2 (IGFBP2), which protects IGF1 from degradation but restricts its receptor interaction. High impact publications has shown IGFBP2 as an astrocyte-derived protein crucial for plasticity but its precise cellular origin, localization and role in plasticity remain unclear.

AIM(S): We aim to determine the cellular source and synaptic localization of IGFBP2 and evaluate its role in IGF1-dependent structural plasticity.

METHOD(S): Immunofluorescence was performed in dissociated neuronal cultures, hippocampal slices and organotypic hippocampal cultures to determine IGFBP2 cellular origin. Colocalization with synaptic markers was analyzed to assess enrichment at excitatory synapses. Synaptoneurosomes were isolated and analyzed by Western blotting to show IGFBP2 presence within synapses as well as astrocytic perisynaptic processes. Proteolytic processing of IGFBP2 by MMP-9 was examined using stimulated synaptoneurosomes in the presence or absence of MMP-9 inhibitors. Two-photon imaging combined with glutamate uncaging was used to assess structural long-term potentiation (sLTP) and to test involvement of IGFBP2 and MMP-9 in IGF1R activation.

RESULTS: We show that IGFBP2 is astrocytic protein and localizes in proximity to excitatory synapses. We identify IGFBP2 as crucial for spine volume enlargement and IGF1R activation during sLTP. Moreover, we confirm IGFBP2 cleavage by MMP-9 and their involvement in described extracellular system.

CONCLUSIONS: Astrocyte-derived IGFBP2 localizes on synapses and is required for IGF1R-dependent structural plasticity. MMP-9-mediated processing of IGFBP2 regulates local IGF1 availability at synapses.

FINANCIAL SUPPORT: National Science Centre, Poland grant: 2023/51/B/NZ4/02135.

## S2P16. MATERNAL MONOSACCHARIDE EXPOSURE IS ASSOCIATED WITH MALE-SPECIFIC VENTRAL HIPPOCAMPAL PLASTICITY

Kacper Witek, Karolina Wydra, Małgorzata Filip

*Department of Drug Addiction Pharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Early-life exposure to dietary monosaccharides can shape neurodevelopmental trajectories and activity-dependent plasticity within limbic circuits of the offspring. However, the long-term consequences for ventral hippocampal plasticity remain insufficiently defined. Our previous studies using the same maternal dietary model demonstrated anxiety-like behavioral alterations in male offspring and dorsal–ventral reorganization of hippocampal neurodevelopment following maternal fructose exposure.

AIM(S): We investigated whether maternal isocaloric glucose or fructose intake programs molecular pathways of synaptic plasticity in the ventral hippocampus during adolescence and young adulthood.

METHOD(S): Male Wistar offspring were evaluated at adolescence (PND28) and young adulthood (PND63) after perinatal maternal exposure to isocaloric control, glucose, or fructose diets. Ventral hippocampal expression of melanocortin-4 receptor (Mc4r), activity-regulated cytoskeleton-associated protein (Arc), brain-derived neurotrophic factor (Bdnf), and neuronal PAS domain protein 4 (Npas4) was quantified at the mRNA level by RT-qPCR and at the protein level by Western blotting.

RESULTS: Adolescence was characterized by limited and dissociated molecular alterations. In contrast, young adulthood revealed a coordinated plasticity profile defined by increased MC4R and ARC protein abundance together with reduced NPAS4 expression, with the strongest and most consistent effects observed in fructose-exposed offspring, while BDNF levels remained largely unchanged. These findings indicate selective remodeling of activity-dependent signaling rather than generalized neurotrophic activation.

CONCLUSIONS: Maternal monosaccharide exposure is associated with a male-specific molecular signature of ventral hippocampal plasticity converging on the MC4R–ARC–NPAS4 axis, providing a plausible mechanistic substrate for previously observed behavioral vulnerability.

FINANCIAL SUPPORT: This study was supported by the research grant UMO-2023/49/N/NZ1/03606 from the National Science Centre (Kraków, Poland) to Kacper Witek.

## S2P17. CIRCADIAN PLASTICITY OF SYNAPSES IN THE VISUAL SYSTEM OF INSECTS

Alice Ballabio, Elżbieta Pyza

*Department of Cell Biology and Imaging, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Circadian rhythms are ~24-hour cycles that regulate physiology and behaviour. They are generated by cell-autonomous molecular clocks and synchronized by environmental cues, like light–dark cycles. In Drosophila melanogaster, the circadian system consists of central pacemaker neurons and peripheral clocks. The visual system (namely the lamina, the first optic lobe neuropil) shows circadian plasticity in synaptic number and morphology of neurons and glia. R1–R6 photoreceptors function as peripheral clock neurons and form tetrad synapses with interneurons, glial cells, and amacrine cells in the lamina. These synapses undergo daily structural changes that relate with locomotor activity rhythms.

AIM(S): This project examines how external cues (light and temperature) and internal factors (oxidative stress) regulate circadian synaptic plasticity of tetrad synapses in the lamina. It also seeks to identify molecular pathways underlying these cyclic structural changes.

METHOD(S): BRUCHPILOT (BRP), a presynaptic active zone protein, served as a marker of tetrad presynaptic structure. Its levels were quantified using immunohistochemistry and confocal microscopy in various light conditions: standard (LD 12:12), short (LD 8:16), long (LD 16:8) photoperiods, constant darkness and light. To complement anatomical data, bulk transcriptomic profiling will be conducted at two circadian time points (morning activity peak and midnight sleep), followed by differential gene expression analysis.

RESULTS: Synapse number and size peak in the morning and evening and decline at midday and midnight. BRP levels follow the same pattern: the morning peak is light-dependent, while the evening peak persists in constant darkness, indicating endogenous clock control.

CONCLUSIONS: Photoreceptors integrate environmental and physiological signals with circadian timing to regulate synaptic plasticity. Ongoing molecular analyses will further clarify the genetic pathways linking circadian rhythms to structural synaptic remodeling in the visual system.

FINANCIAL SUPPORT: INCITE (Z/heu/00049).

## S2P18. SYSTEM-LEVEL EFFECTS OF NEUROSTEROIDS: FROM RECEPTOR MODULATION TO BEHAVIORAL OUTCOMES

Marek Kuruc

*Laboratory of Cellular Neurophysiology, Institute of Physiology, Czech Academy of Sciences, Prague, Czechia*

INTRODUCTION: Brain function relies on tightly regulated excitatory and inhibitory signaling. NMDA and AMPA receptors mediate excitation, while GABA_A receptors provide synaptic inhibition. Disruption of this balance contributes to neuropsychiatric disorders. Neurosteroids fine-tune these receptors and can rapidly strengthen or weaken their activity, yet their behavioural consequences remain poorly understood. The zebrafish has emerged as a model for brain disorders, as its neurotransmitter systems are highly conserved with mammals.

AIM(S): Here, we investigated the neurosteroid 5β-pregnan-20-deoxo-3α-yl-sulfate (PAdO-S) across molecular, synaptic, and behavioural scales to link receptor modulation with system-level outcomes.

METHOD(S): Using whole-cell patch-clamp electrophysiology, we examined PAdO-S actions on recombinant rat and zebrafish NMDA receptors expressed in HEK293 cells and on synaptic transmission in rat hippocampal autaptic cultures. Behavioural effects were assessed by measuring spontaneous locomotor activity in zebrafish larvae.

RESULTS: PAdO-S inhibited recombinant rat and zebrafish NMDA receptors with comparable potency in a dose-dependent manner (0,1 – 10 μM), supporting the validity of our model. At synapses, PAdO-S (10 μM) reduced GABA_A receptor–mediated IPSCs. AMPA receptor–mediated EPSCs were potentiated by 41,4%, while paired-pulse ratio decreased by 43,4% (10 μM), indicating enhanced presynaptic glutamate release. Behavioural benchmarks were established using selective receptor blockers: the GABA_A antagonist picrotoxin (10–30 μM) triggered seizure-like activity in larvae, whereas the NMDA receptor blocker memantine (10–100 μM) reduced locomotion. By contrast, PAdO-S increased locomotor activity without inducing aberrant movement (1–10 μM).

CONCLUSIONS: PAdO-S exerts pre- and postsynaptic actions, and the balance between these effects shapes circuit-level and behavioural outcomes. Exploring these mechanisms may clarify the therapeutic actions of neurosteroids in clinical use and guide future work.

FINANCIAL SUPPORT: This work was supported by the Czech Science Foundation (GAČR), grant no. 23-04922S.

## S2P19. VISFATIN REGULATES GNRH SECRETION BY MOUSE HYPOTHALAMIC GT1-7 CELLS *VIA* A PKC-DEPENDENT SIGNALLING PATHWAY

Natalia Respekta-Długosz[1,2], Zofia Żurek[1], Aleksandra Greggio[1,2], Wiktoria Kowalczyk[1], Agnieszka Rak[1]

[1] *Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*

[2] *Doctoral School of Exact and Natural Science, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Visfatin, an adipocytokine involved in the metabolic homeostasis, has been implicated in reproductive regulation; however, its direct effects on hypothalamic gonadotropin-releasing hormone (GnRH) neurons remain unclear.

AIM(S): We hypothesized that visfatin directly modulates GnRH neuronal activity by stimulating GnRH secretion via a protein kinase C (PKC)-dependent signaling pathway. Therefore, the aims of this study were: (i) to confirm the expression of visfatin in mouse hypothalamic GnRH neurons, (ii) to determine whether visfatin directly affects GnRH secretion and expression in GT1-7 cells, and (iii) to elucidate the involvement of PKC-dependent signaling in visfatin-induced modulation of GnRH neuronal activity.

METHOD(S): In vitro experiments were performed using the mouse hypothalamic GnRH neuronal GT1-7 cell line. Firstly, visfatin expression was confirmed at the mRNA level by RT-qPCR and showed co-localization with GnRH by immunocytochemistry. Secondly, cells were treated with visfatin (10 or 100 ng/mL) for 15 or 30 min and then GnRH secretion was quantified by ELISA, protein expression by Western blot, and mRNA levels by RT-qPCR. Finally, the involvement of PKC signaling in the regulation of GnRH level was assessed using the pharmacological inhibitor Bisindolylmaleimide I (2 nM). Statistical analysis employed Student's t test ($n \geq 4$; $p < 0.05$).

RESULTS: Visfatin significantly stimulated GnRH secretion at both time points, accompanied by increased GnRH protein levels. Pharmacological inhibition of PKC markedly attenuated visfatin-induced GnRH secretion.

CONCLUSIONS: In conclusion, visfatin acts as a direct modulator of GnRH neuronal activity at the secre-

tory level via PKC-dependent signaling. These findings provide new evidence linking adipocytokine signaling with hypothalamic control of reproduction and highlight visfatin as a potential mediator of metabolic–reproductive crosstalk.

FINANCIAL SUPPORT: National Science Centre (project no. 2021/42/E/NZ4/00088).

## S2P20. ADROPIN EXPRESSION AND *IN VITRO* EFFECT ON VIABILITY IN THE PORCINE ANTERIOR PITUITARY CELLS DURING ESTROUS CYCLE

Aleksandra Greggio[1,2], Natalia Respekta-Długosz[1,2], Agnieszka Rak[1]

*[1] Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*
*[2] Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Adropin is a hormone discovered in 2008 that is expressed in the brain, liver, kidneys, lungs, muscles, and adipose tissue; its expression is strongly associated with the body's metabolic status and can positively affect glucose metabolism and insulin resistance, among other processes. The body's metabolic status has a strong influence on reproduction; however, there are limited studies on the role of adropin in the hypothalamic–pituitary axis.

AIM(S): Therefore, in this study, we focused on describing the role of adropin in porcine pituitary cells.

METHOD(S): First, we examined the expression of adropin and its receptor, GPR19, at both the gene and protein levels (RT-qPCR, Western blot) in the anterior pituitary of female pigs across different phases of the estrous cycle (days 2–3, 10–12, 14–16, and 17–19). Additionally, we examined the effect of adropin (0.1, 1, 10, and 100 nM) after 24 h on the viability of anterior pituitary cells collected from different phases of the estrous cycle (alamarBlue assay).

RESULTS: Our results, for the first time, demonstrate the expression of adropin in female pigs throughout the estrous cycle. Furthermore, we showed that adropin exerts a downregulating effect on the viability of anterior pituitary cells across all phases of the estrous cycle.

CONCLUSIONS: These findings provide a foundation for further analyses of the precise effects of metabolism-related hormones on pituitary cells and may contribute to a better understanding of how metabolic hormones influence reproductive processes.

FINANCIAL SUPPORT: Budget of Institute of Zoology and Biomedical Research, Jagiellonian University.

## S2P21. ROLE OF GLIAL PITUICYTES IN NEUROHYPOPHYSEAL SYNAPTIC MORPHOGENESIS

Naveen Nedunchezhian[1], Athul R. Ramesh[1], Natalia Kowalewska[1], Sebastian Pęcherz[1], Felix Klenner[2], Gilbert Weindinger[2], Savani Anbalagan[1]

*[1] Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland*
*[2] Institute of Biochemistry and Molecular Biology, Ulm University, Ulm, Germany*

INTRODUCTION: Neurohypophysis (posterior pituitary) is a major neuroendocrine interface in the brain through which water homeostasis is maintained. Neurohypophysis majorly consists of glial pituicytes, neuropeptides oxytocin- and vasopressin-loaded loaded synapses and permeable capillaries.

AIM(S): We recently identified that pituicyte-derived secreted factor can regulate neurohypophyseal neurovascular morphogenesis. However, the role of other secreted factors expressed in the neurohypophysis in neurovascular morphogenesis is unknown

METHOD(S): To achieve this goal, we have employed pharmacological and genetic perturbations to investigate the roles of candidate molecules that regulate neurohypophyseal synapse morphogenesis.

RESULTS: We found that pharmocological and genetic pertubation of Wnt signalling can regulate neurohypophysis axonal and synaptic morphogenesis. Through transcriptomic datamining, we have identified some Wnt regulators as potential candidate genes and signaling pathways that govern NH-specific axonbal and synaptic morphogenesis

CONCLUSIONS: Our studies of the glial pituicytes are expected to reveal novel players in the development of a key neuroendocrine interface conserved in vertebrates.

FINANCIAL SUPPORT: SONATA-BIS 2020/38/E/NZ3/00090.

## S2P22. CHANGES IN EXCITATORY TRANSMISSION TO SST INTERNEURONS IN THE MEDIAL PREFRONTAL CORTEX IN MICE SUBJECTED TO EARLY-LIFE MATERNAL SEPARATION

Aleksandra Sawicka, Karolina Bogaj, Julia Kokosza, Joanna Urban-Ciećko

*Laboratory of Electrophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Maternal separation, as a serious early-life stressor (ELS), has been recognized as a high risk factor that can lead to many psychiatric disorders. Disruptions in the medial prefrontal cortex (mPFC) are strictly related to those disorders. However, little is known about exact effects of ELS on neuronal function in mPFC. GABAergic interneurons are inhibitory neurons that are involved in basic brain functions and maintaining excitation/inhibition balance (E/I balance) in the neuronal networks. Somatostatin-expressing (SST) interneurons are a subtype of GABAergic neurons, which control the output of cortical pyramidal neurons.

AIM(S): The aim of the study was to dissect the effects of maternal separation on electrophysiological properties of SST interneurons in the mouse mPFC.

METHOD(S): Transgenic mice with fluorescently labeled SST interneurons were subjected to maternal separation between the 1st and the 20th postnatal day, for 3 hours daily. Mice from control groups were left undisturbed with their dams until weaning. Over the next few days behavioral tests were performed on both groups. After behavioral testing, whole-cell patch-clamp recordings were performed in acute brain slices. To measure E/I balance, miniature excitatory and inhibitory postsynaptic currents (mEPSC/mIPSC) were recorded in SST interneurons.

RESULTS: Statistical analysis revealed changes in mEPSCs but not in mIPSCs in SST interneurons after maternal separation; the amplitude of mEPSCs was significantly lower than in the control group, whereas the mEPSC frequency did not change between the groups.

CONCLUSIONS: These results suggest that ELS related to maternal separation weakens excitatory transmission to SST interneurons. This study provides an insight into the effects of the early-life maternal separation on the function of SST interneurons in the mPFC of offspring.

FINANCIAL SUPPORT: This study was supported by National Science Centre Poland, OPUS grant no. 2025/57/B/NZ5/03816 to JUC, OPUS grant no. 2020/39/B/NZ4/01462 to JUC.

## S2P23. RELAXIN-3 AND OXYTOCIN SIGNALING IN THE VENTRAL DENTATE GYRUS: NEUROCHEMICAL CHARACTERIZATION AND OPPOSING ROLES IN STRESS AND ANXIETY

Kinga Przybylska[1,2], Aleksandra Trenk[1], Anna Guguła[1], Aleksandra Nogaj[1,2], Gabriela Stopka[1,2], Mohammed Akhter Hossain[3], Andrew L. Gundlach[3], Anna Błasiak[1]

*[1] Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

*[2] Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

*[3] The Florey Institute of Neuroscience and Mental Health, and Florey Department of Neuroscience and Mental Health, The University of Melbourne, Melbourne, Australia*

INTRODUCTION: The ventral hippocampus (vHPC) plays a critical role in regulating stress and anxiety, with interneuron dysfunction implicated in anxiety-related disorders. The vHPC receives substantial innervation from the nucleus incertus (NI), the primary source of relaxin-3 (RLN3) in the rat brain. RLN3 signaling in vHPC has been shown to promote anxiety and social avoidance. In contrast, oxytocin (OXT) signaling within vHPC enhances social bonding and reduces stress, highlighting opposing neuromodulatory effects of these neuropeptides. However, their interaction within this structure remains poorly understood.

AIM(S): This study aimed to characterize the neurochemical profile and functional connectivity of the NI–vHPC pathway and determine how RLN3/RXFP3 and OXT/OXTR signaling interact to modulate vHPC neuronal activity.

METHOD(S): Anatomical, molecular, and electrophysiological approaches were employed in rats, including viral based tract-tracing, immunohistochemistry, HiPlex in situ hybridization (ISH), and ex vivo multi-electrode array (MEA) recordings.

RESULTS: Immunohistochemistry and viral tract-tracing revealed dense RLN3-positive and NI-originating fibers in the ventral dentate gyrus (vDG), particularly within the hilus. ISH demonstrated that RXFP3 and OXTR mRNAs are expressed in GABAergic (vGAT1-positive) neurons, including subpopulations expressing somatostatin and/or parvalbumin. Notably, we also observed co-expression of RXFP3, OXTR, and KCNQ2 mRNA in individual vDG neurons. MEA recordings revealed opposing effects of RLN3 and OXT on vDG neural activity, with RLN3 exerting inhibitory and OXT excitatory effects. Fur-

thermore, the blockade of KCNQ channels with XE991 reduced both of these responses.

CONCLUSIONS: These findings demonstrate that RLN3 and OXT directly modulate vDG neuronal activity and exert antagonistic effects via common molecular effector, KCNQ channels. This interaction may represent a molecular substrate underlying the regulation of stress- and anxiety-related behaviors.

FINANCIAL SUPPORT: National Science Centre Poland: UMO-2023/49/B/NZ4/01885, MiniGrant2023 ID.UJ.

## S2P24. SINGLE EXPOUSURE TO VANADIUM INDUCES DOSE-DEPENDENT PROTEOMIC CHANGES INVOLVING STRESS, IMMUNOLOGIC AND NEURONAL RESPONSES IN THE PREFRONTAL CORTEX OF C57BL/6J MICE

Aleksandra Karska, Przemysław Mielczarek, Łukasz Gąsior, Katarzyna Stachowicz

*Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Vanadium compounds are known to modulate cellular signaling and metabolic pathways, but their dose-dependent effects in the brain remain poorly characterized. In particular, little is known about how acute exposure to vanadium affects protein expression in the prefrontal cortex. This is vital because such changes may influence memory and mental health.

AIM(S): The aim of this study was to analyze the effects of a single exposure to vanadium at doses of 2 mg/kg and 20 mg/kg on proteomic changes in the prefrontal cortex (PFC) of C57BL/6J mice.

METHOD(S): C57Bl/6J mice were acutely injected with sodium orthovanadate dissolved in water at doses of 2 mg/kg (V2) and 20 mg/kg (V20) (i.p.). High-throughput proteomic analysis was used to detect changes in protein expression in the prefrontal cortex (pFc). The pFc was selected according to Paxinos and Franklin, 2001.

RESULTS: Compared to the vehicle group, 22 proteins were differentially regulated in the V2 group (e.g., Mrfap1, Pla2g4a, Slc30a3, and down-regulated Rragd) and 26 proteins in the V20 group (Fkbp1b, Calml3, Ighg2b). The low dose of V induced moderate changes in pFC proteins, influencing mainly mitochondrial metabolism, synaptic function, and post-transcriptional regulation. These changes suggest an adaptive response. In contrast, the high dose of V (20 mg/kg) induced a stronger, mainly regulatory profile involving mitochondrial function, proteostasis, cellular stress responses, and neuronal signaling – indicating an increased cellular demand for homeostasis maintenance.

CONCLUSIONS: Together, these findings demonstrate that vanadium treatment induces distinct, dose-dependent proteomic signatures. The changes range from subtle metabolic and neuronal modulation at low doses to extensive metabolic and stress-related remodeling at high doses. This study provides insight into the molecular mechanisms underlying the biological response to increasing vanadium intensity. It also highlights key pathways potentially involved in neuronal and metabolic regulation.

FINANCIAL SUPPORT: The study was conducted thanks to funding from the statutory funds of the Maj Institute of Pharmacology of the Polish Academy of Sciences.

## S2P25. INTRACELLULAR SIGNALING PATHWAYS INVOLVED IN TRKB-DEPENDENT REGULATION OF PYRAMIDAL NEURONS IN THE MEDIAL PREFRONTAL CORTEX

Anna Wierucka[1], Przemysław Kurowski[1,2]

[1] *Chair and Department of Pharmacotherapy and Pharmaceutical Care, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland*

[2] *Laboratory of Physiology and Pathophysiology, Centre for Preclinical Research and Technology, Medical University of Warsaw, Warsaw, Poland*

INTRODUCTION: A reduction in brain-derived neurotrophic factor (BDNF) levels negatively affects TrkB receptors, ultimately disrupting the activity of downstream signaling pathways. Decreased BDNF expression has been observed in several neuropsychiatric disorders, including depression. Key contributing mechanisms of depression include oxidative stress and neuroinflammation, which lead to dysfunction within the BDNF/TrkB signaling pathway. However, the intracellular signal transduction pathways regulated by TrkB receptors in pyramidal neurons of the medial prefrontal cortex (mPFC) are not fully understood.

AIM(S): The aim of this study was to identify intracellular signal transduction pathways involved in TrkB-dependent modulation of layer V pyramidal neurons in the mPFC, with particular focus on protein kinase A (PKA), phosphoinositide 3-kinase (PI3K), and protein kinase C (PKC).

METHOD(S): Whole-cell current-clamp recordings were performed in synaptically isolated pyramidal neurons in acute mPFC slices obtained from young male rats.

RESULTS: Pharmacological activation of TrkB receptors, using HIOC, induced significant changes in neuronal membrane potential. Co-application of HIOC with a selective TrkB antagonist abolished these effects, confirming the specificity of the agonist. Furthermore, the use of selective inhibitors revealed that PKA, PI3K, and PKC contribute to TrkB-dependent regulation of membrane excitability.

CONCLUSIONS: These findings indicate that TrkB receptors regulate the excitability of pyramidal neurons in the mPFC through modulation of PKA, PI3K, and PKC signaling pathways. The results highlight potential molecular targets for the development of novel antidepressant therapies.

FINANCIAL SUPPORT: This work was supported by the Ministry of Science and Higher Education, Poland (grant no. SKN/SP/630586/2025).

## S2P26. A MATRIX-DERIVED PEPTIDE THAT CALMS BRAIN IMMUNE CELLS: EXPLORING GLYCINE–HISTIDINE–LYSINE–COPPER (GHK–CU) AS A REGULATOR OF MICROGLIAL FUNCTIONS

Mitchell Beckedorf[1], Tyler J. Wenzel[2], Andis Klegeris[1]

[1] *Faculty of Science, University of British Columbia Okanagan Campus, Kelowna, Canada*

[2] *College of Medicine, University of Saskatchewan, Saskatoon, Canada*

INTRODUCTION: Alzheimer's disease (AD) is the leading cause of dementia worldwide and is characterized by amyloid-β and tau accumulation with chronic neuroinflammation. As the resident immune cells of the central nervous system (CNS), microglia play a key role in disease progression and are important therapeutic targets. Matrikines are bioactive peptides released from the extracellular matrix during tissue remodeling that regulate immune signaling and homeostasis across multiple tissues and have been studied mainly in the periphery. Emerging evidence indicates that matrikines are also released in the CNS, where they contribute to homeostasis and neuroimmune regulation. The copper-binding tripeptide glycine–histidine–lysine (GHK) is a matrikine with established anti-inflammatory and regenerative actions in the periphery, yet its effects in the brain remain poorly understood.

AIM(S): To evaluate the effects of GHK and its copper complex, GHK-Cu, on microglia-like cells under oxidative stress and neuroinflammation relevant to AD.

METHOD(S): BV-2 murine microglial cells and differentiated HL-60 human promyelocytic cells were treated with GHK, GHK-Cu, or $CuSO_4$ prior to stimulation with lipopolysaccharide or interferon-γ. Inflammatory mediators, nitric oxide, and reactive oxygen species were quantified. Phagocytosis and morphological changes were assessed by fluorescence microscopy.

RESULTS: GHK-Cu reduced IL-6 and nitric oxide in activated BV-2 microglia and suppressed reactive oxygen species in primed HL-60 cells. It increased phagocytic activity and decreased cell circularity, indicating reduced reactive morphology. Copper-free GHK had no effect, while $CuSO_4$ induced cytotoxicity. GHK-Cu protected microglia from inflammatory toxicity but did not significantly reduce their neurotoxicity.

CONCLUSIONS: GHK activity in the CNS depends on copper complexation. By suppressing pro-inflammatory signaling and enhancing phagocytosis, GHK-Cu may restore microglial homeostasis and is a promising endogenous modulator of neuroinflammation.

FINANCIAL SUPPORT: NSERC Canada; J. Brown and Family Alzheimer's Disease Research Foundation.

## S2P27. BEHAVIOURAL IMPAIRMENT OF ADULT SPRAGUE-DAWLEY RATS AFTER 4-VESSEL GLOBAL ISCHEMIA

Kinga Nazaruk, Kinga Szydlowska, Dorota Nowicka, Katarzyna Lukasiuk

*Department of Epileptogenesis, Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Epilepsy is a common neurological disorder characterized by the occurrence of unprovoked seizures.

AIM(S): In our study, we aim to investigate the behavioural changes in the rat model of epilepsy following four-vessel occlusion ischemia.

METHOD(S): We performed a battery of behavioural tests on adult Sprague-Dawley rats, before and after the ischemia.

RESULTS: The Open Field test and Hyperexcitability test did not reveal any significant changes between the Sham and Ischemic groups. In the New Object Recogni-

tion test Ischemic animals visited the novel object and the surrounding area less frequently. They also moved shorter distances and showed less interest in the new object, in contrast to control animals, which climbed on it. The Elevated Plus Maze test showed that ischemic animals spend more time in the closed arms and travel less than Sham animals. In the Social Interactions Test, both groups of animals presented the correct behaviour by spending a longer time in a compartment with a new, unknown rat than in the compartment with the familiar rat. Control animals presented more exploratory behaviour when moving around the cage, and ischemic animals spent more time in the empty area.

CONCLUSIONS: The results of behavioural tests indicate increased anxiety in ischemic animals.

FINANCIAL SUPPORT: NCN Opus-22.

## S2P28. TARGETING POST-ISCHEMIC NEUROINFLAMMATION WITH PAPE-1 BEYOND THE ACUTE THERAPEUTIC WINDOW

Andrzej Łach[1], Bernadeta A. Pietrzak-Wawrzyńska[1], Karolina Przepiórska-Drońska[1,2], Wiktoria Płonka[1], Agnieszka Wnuk[1]

*[1] Department of Pharmacokinetics and Drug Metabolism, Team III, Maj Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland*

*[2] Department of Experimental Neuroendocrinology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Therapeutic options for ischemic stroke remain limited, particularly beyond the acute intervention window and secondary injury mechanisms such as neuroinflammation are poorly targeted by current treatments. To combat those limitation we researched a novel compound Pathway Preferential Estrogen-1 (PaPE-1), designed to selectively activate non-nuclear estrogen receptor (ER) signaling while minimizing classical hormonal detrimental effects associated with non-specific ERs activation.

AIM(S): This study investigated the effects of PaPE-1 on post-ischemic inflammatory responses and examined whether modulation of retinoid X receptor (RXR) isoforms expression contributes to its neuroprotective actions.

METHOD(S): Mouse primary neocortical neurons and a human microglial cell line were subjected to ischemia followed by PaPE-1 treatment during reoxygenation to model delayed intervention. Inflammatory mediators (interleukins, Cox2, Nlrp3) were quantified using qPCR and ELISA. We have also assessed the neuronal expression of RXRα, RXRβ, and RXRγ and inflammation-associated microRNAs. Microglial proliferation and viability were evaluated using BrdU incorporation, calcein staining, and morphological analysis.

RESULTS: PaPE-1 significantly reduced ischemia-induced pro-inflammatory signaling including IL1β, IL10, COX2, miR-19a, miR-130a and miR-132. Ischemia decreased neuronal RXRα isoform expression, and PaPE-1 was able to attenuate these changes. Moreover, PaPE-1 normalized microglial activation, as indicated by reduced BrdU incorporation and decreased microglial cell area.

CONCLUSIONS: Selective activation of non-nuclear ER signaling by PaPE-1 attenuates post-ischemic inflammation and supports neuronal resilience, working on both genomic and proteomic levels. Regulation of microglia activation together with normalization of neuronal RXR isoforms expression, especially RXRα, may contribute to reduced secondary inflammatory injury, posing PaPE-1 as a promising therapeutic tool for ischemic stroke beyond the acute phase.

FINANCIAL SUPPORT: National Science Centre of Poland, grant number 2021/43/D/NZ7/00633.

## S2P29. SEX-SPECIFIC EFFECTS OF VOLUNTARY WHEEL RUNNING ON INFLAMMASOME PROFILE IN THE FRONTAL CORTEX OF ADULT MICE TREATED WITH METH

Natalia Pondel[1], Mateusz Smolarz[1], Andrzej Małecki[1], Marta Nowacka-Chmielewska[1], Michał Toborek[1,2]

*[1] Institute of Physiotherapy and Health Sciences, Academy of Physical Education, Katowice, Poland*

*[2] Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, USA*

INTRODUCTION: Physical activity (PA) is widely associated with many health benefits e.g. due to effects on brain function. PA has been proposed as a behavioral intervention to counteract the neurotoxic effects of methamphetamine (METH). METH is one of the most popular psychostimulants in the world which contributes to neuroinflammation.

AIM(S): Here, we evaluate if different levels of PA affects inflammasome profile in the frontal cortices of adult female and male mice exposed to METH.

METHOD(S): Female (n=48) and male (n=48) C57BL/6 adult mice were divided into a control group injected with saline (Veh) and mice exposed for 5 days to METH. Then, mice were subjected to 14-days voluntary wheel

running (VWR groups) or kept sedentary (SED). In VWR cohorts PA levels were identified post running period, based on the total distance covered, creating VWR-L, VWR-M and VWR-G groups (low-, moderate-, and good-runners, respectively). At the end of the experiment, cortical samples were collected. We evaluated the level of inflammasome proteins (NLRP1, NLRP3, NLRC4, pro-IL18 and IBA1) by using Western blot analysis.

RESULTS: We observed the significant effect of sex factor for distance covered and time spent in the wheel, showing that females were more active than males. The effect of PA was more pronounced in females, which was manifested in NLRC4 and pro-IL18, and showed a tendency in IBA1 and NLRP1. In females, the PA x METH interaction was revealed for IBA1. The effect of METH was noticed only in the level of pro-IL18 in females. In males, we observed the significant effect of the PA on the expression of IBA1 and the trend in NLRP3. Moreover, we found an interaction between PA and METH on the protein level of NLRP3.

CONCLUSIONS: The results demonstrate distinct inflammasome protein responses in the cortex on PA with different levels in METH-treated adult mice in sex-dependent manner.

FINANCIAL SUPPORT: The study was supported by the National Science Centre (NSC) grant 2019/35/B/NZ7/03155.

## S2P30. ANTI-APOPTOTIC POTENTIAL OF CLASSICAL AND NOVEL ANTI-INFLAMMATORY COMPOUNDS – *IN VITRO* STUDY IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

Danuta Jantas[1], Aleksandra Mąsior[1], Piotr Chmielarz[1], Ryszard Bugno[2], Grzegorz Kreiner[1]

[1] *Department of Brain Biochemistry, Maj Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland*

[2] *Department of Medicinal Chemistry, Maj Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: MIP001 is novel anti-inflammatory and analgesic drug with favourable safety profile even at high doses. In our previous studies we showed a neuroprotective potential of MIP001. This compound, but not classical anti-inflammatory agent ibuprofen (Ibu), showed a higher protective efficacy in apoptotic (MPP+), than oxidative stress-based (H2O2 and 6-OHDA) neuronal cell damage models.

AIM(S): In order to further investigate neuroprotective potency of MIP001 in comparison to the effect of Ibu, we employed two models of apoptosis: i) based on activation of intracellular (staurosporine, St) and ii) extracellular (doxorubicin, Dox) apoptotic pathways in undifferentiated (UN-) and retinoic acid (RA)-differentiated SH-SY5Y cells.

METHOD(S): The neuroprotective effects were measured by biochemical assays (MTT reduction and LDH release assays), microscopic imaging and evaluation of apoptosis (caspase-3 activity) and necrosis (propidium iodide staining, PI) markers.

RESULTS: We demonstrated that MIP001 (at concentrations 10-100 microM) but not Ibu partially attenuated the cell damage induced by Dox and this effect was observed only in RA-SH-SY5Y cells. However this was not associated with attenuation of caspase-3 activity nor lowering of the number of necrotic cells. We did not find any protection of MIP001 and Ibu against cell damage induced by St in both studied cell phenotypes.

CONCLUSIONS: Our results evidenced a neuroprotective potential of MIP001 in neuronally differentiated SH-SY5Y cells against apoptotic cell damage induced extracellularly probably via inhibition of caspase-3 independent mechanisms, which need further investigation.

FINANCIAL SUPPORT: The study was supported by a programme coordinated by the Medical Research Agency, co-financed by the European Union under the NextGeneration EU initiative, within the framework of the National Recovery Plan, Component D, Investment D3.1.1 (project no. 2024/ABM/03/KPO/KPOD.07.07-IW.07-0173/24-00).

## S2P31. THE RETINAL LANDSCAPE WITHOUT MICROGLIA: INVESTIGATING THE IMPACT OF MICROGLIA DEPLETION DURING DEVELOPMENT ON THE MORPHOLOGY OF THE RETINA

Barbara Siewert[1,2], Natalie Oezgen[1], Sandra Siegert[1]

[1] *Institute of Science and Technology Austria (ISTA), Klosterneuburg, Austria*

[2] *Gdański Uniwersytet Medyczny, Gdańsk, Poland*

INTRODUCTION: Microglia are the resident immune cells of the central nervous system (CNS) and play a key role in maintaining the normal function of the retina and brain. Recent studies suggest that of microglia may regulate synaptic structure and function by pruning functionally weak synapses. The retinal circuit is infiltrated by microglia by embryonic (E) day 11.5, where they reside in the emerging inner plexiform layer (IPL)

and outer plexiform layer (OPL) during development and throughout adulthood. However, in what ways microglia regulate neuronal circuit formation during development and signal transmission in the retina remains unknown.

AIM(S): This study aimed to investigate whether continuous depletion of microglia during embryonic and early postnatal development affects the structural organization and morphology of the retina.

METHOD(S): Microglia were depleted using the CSF1R inhibitor PLX5622 from E17.5 hence during embryonic and early postnatal development. Retinal tissue was analyzed using immunohistochemistry and laser confocal microscopy. Quantitative image analysis by Imaris was performed to assess retinal layer organization, thickness, structure shape and distribution, and synaptic markers in microglia-depleted and control retinas.

RESULTS: Morphological analysis showed no significant differences between PLX-treated and control retinas in layer thickness or in the sphericity and volume of ribbon synapses. However, there seems to be a trend (p-value: 0.079) towards lower number of ribbon synapses in the OPL of PLX-treated retinas (788 ± 144) compared to controls (994 ± 49).

CONCLUSIONS: Although microglia are implicated in synaptic refinement, our preliminary findings suggest that their depletion does not overtly disrupt the gross morphological organization of the developing retina. Future studies should investigate potential subtle circuit-level or functional consequences and explore compensatory mechanisms that may preserve retinal structure in the absence of microglia.

## S2P31. LIVER-EXPRESSED ANTIMICROBIAL PEPTIDE 2 AS A MODULATOR OF INFLAMMATION INDUCED IN HUMAN MICROGLIAL CELLS-HMC3: AN *IN VITRO* STUDY

Agnieszka Górka

*Department of Animal Physiology and Biochemistry, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Poznań, Poland*

INTRODUCTION: With the advancement of research on the nervous system, increasing attention has been paid to the role of glial cells in maintaining central nervous system (CNS) homeostasis and regulating immune responses. Microglia, the primary immune cells of the CNS, protect neural tissue against toxins and pathogens. However, their excessive activation can lead to chronic inflammation, which underlies many neurodegenerative disorders. Natural antimicrobial peptides (AMPs) have recently attracted interest due to their antibacterial and immunomodulatory properties. One such peptide is liver-expressed antimicrobial peptide 2 (LEAP2), known for its activity against Gram-positive bacteria. In addition to its antimicrobial function, LEAP2 acts as an antagonist of the ghrelin receptor (GHSR), which is involved in appetite regulation and immune modulation. Owing to its multifunctional properties, LEAP2 may serve as a regulator of inflammatory responses in microglial cells.

AIM(S): The aim of this study was to evaluate the effect of LEAP2 on lipopolysaccharide (LPS)-induced inflammation in Human microglial cells (HMC3).

METHOD(S): The study employed qPCR, MTT, and BrdU assays, as well as immunofluorescence (IF), to analysed the expression of Caspase 3 (CASP3), Interleukin 6 (IL-6), Tumor Necrosis Factor α (TNFα), and growth hormone secretagogue receptor (GHSR) genes, along with cell viability and proliferation.

RESULTS: LEAP2 treatment modulated the expression of pro-inflammatory cytokine genes and improved cell viability and proliferation in activated HMC3 cells. IF analysis confirmed the presence of LEAP2, ghrelin, and GHSR in the HMC3 cells.

CONCLUSIONS: These findings support the immunomodulatory potential of LEAP2 in neuroinflammation. However, further studies with larger sample sizes and additional neural cell models are required to confirm these results.

## S2P32. INNOVATIVE NEUROPROTECTION: COMMENSAL BIFIDOBACTERIUM EXTRACELLULAR VESICLES PRESERVE BLOOD–BRAIN BARRIER FUNCTION UNDER INFLAMMATORY CONDITIONS

Agnieszka Zabłocka[1], Dominika Kozakiewicz[1], Natalia Małek[2], Dominika Jakubczyk[1], Józefa Macała[1], Sabina Górska[1]

[1] *Laboratory of Microbiome Immunobiology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland*

[2] *Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland*

INTRODUCTION: Age-related alterations of the gut microbiota contribute to systemic inflammation. Circulating pro-inflammatory factors may impair blood–brain barrier (BBB) integrity, promoting central nervous system inflammation, neuronal damage, and progressive cognitive decline. Although the gut–brain axis

is increasingly recognised as a key regulator of brain health, the specific contribution of commensal bacteria and their secreted products remains poorly defined.

AIM(S): This study aimed to characterise bacterial extracellular vesicles (BEV) produced by Bifidobacterium animalis subsp. animalis CCDM 366 (Ba366) and to evaluate the protective effects of Ba366 and its BEV on BBB integrity.

METHOD(S): Human brain microvascular endothelial cells (HBEC-5i) were used as an in vitro BBB model. The effects of Ba366 and BEV were assessed under physiological and inflammatory conditions. Cell viability and proliferation were analysed using MTT assay and Incucyte S3 live-cell imaging. BBB integrity was evaluated by transendothelial electrical resistance and paracellular permeability assays. Transcriptomic analysis was performed to identify changes in gene expression.

RESULTS: Ba366 and BEV were non-cytotoxic to HBEC-5i cells, modulated cell proliferation, reduced paracellular permeability, and enhanced barrier integrity. Ba366 enriched genes associated with cell proliferation and hormonal and neuronal signalling, whereas BEV enriched genes involved in ion transport, neurotransmitter signalling, membrane function, and pathways regulating autophagy and cell growth.

CONCLUSIONS: Ba366 and its BEV modulate BBB integrity and function through complementary molecular mechanisms. In the context of age-associated gut microbiota dysbiosis and systemic inflammation, these findings support the potential of Ba366-derived components to preserve or restore BBB integrity and mitigate neuroinflammatory processes.

FINANCIAL SUPPORT: This work was supported by the Biocodex Microbiota Foundation.

## S2P33. MESSAGES IN EXTRACELLULAR VESICLES: DECODING MIRNA BIOMARKERS IN MULTIPLE SCLEROSIS

Andrėja Strigauskaitė[1,2], Violeta Belickienė[1], Rūta Juozaitienė[3], Gabrielė Dargė[3], Monika Kurgonaitė[4], Renata Balnytė[5], Ieva Masiulienė[5], Paulina Vaitkienė[1], Ugnė Zubrickaitė[3], Dalia Čalnerytė[3], Edita Kuncevičienė[1]

[1] *Laboratory of Molecular Neurobiology, Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania*

[2] *Medical Faculty, Lithuanian University of Health Sciences, Kaunas, Lithuania*

[3] *Faculty of Informatics, Vytautas Magnus University, Kaunas, Lithuania*

[4] *Department of Applied Informatics, Kaunas University of Technology, Kaunas, Lithuania*

[5] *Department of Neurology, Lithuanian University of Health Sciences, Kaunas, Lithuania*

INTRODUCTION: Multiple sclerosis (MS) is a chronic immune-mediated disease of the CNS characterized by neuroinflammation, demyelination, and progressive neurological disability. Despite significant advances in understanding MS pathogenesis, reliable biomarkers for diagnosis or prognosis are still lacking. MicroRNAs (miRNAs), small non-coding regulators of gene expression involved in immune and neuroinflammatory processes, are stable and detectable in circulation – particularly within extracellular vesicles – making them promising candidates for minimally invasive biomarker discovery.

AIM(S): To identify EV derived miRNAs associated with Multiple sclerosis, evaluate their biomarker potential, and explore their relationships with clinical and radiological features.

METHOD(S): Illumina sequencing of EVderived miRNAs from 22 patient samples. Data were analyzed using DESeq2, PCA, sPLSDA, and Elastic Net to identify candidate miRNAs. Selected miRNAs were validated by qPCR in an independent cohort of 47 patient samples, followed by evaluation of clinical and radiological associations.

RESULTS: PCA revealed two clearly separated clusters corresponding to HC and untreated MS patients. Differential expression analysis using DESeq2 identified 13 miRNAs significantly dysregulated in MS compared to HC. Both sPLSDA and Elastic Net modelling highlighted several miRNAs as major contributors to class discrimination. Strong correlations were observed between miR1973p and miR30e3p and multiple clinical parameters, including disease duration, Expanded Disability Status Scale (EDSS) score, lesion localization, and additional diseaserelated measures. Among the top candidates, miR1973p and miR30e3p were selected for validation by qPCR. Validation confirmed a significant upregulation of miR1973p in MS patients relative to healthy controls.

CONCLUSIONS: The significant upregulation of miR1973p in MS highlights its biomarker potential, but larger studies are needed to confirm these preliminary findings.

FINANCIAL SUPPORT: Funding received from the Association “Santakos slėnis” joint research project competition for science and study institutions. P-ST-25-15, Funded by Reasearch Council of Lithuania.

## S2P34. THE IMPACT OF KETOGENIC DIET ON GLIAL SCAR FORMATION AND NEUROINFLAMMATION AFTER TRAUMATIC BRAIN INJURY

Zuzanna Rauk[1,2], Zuzanna Setkowicz[1]

[1] *Laboratory of Experimental Neuropathology, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*
[2] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Traumatic brain injury (TBI) is a disorder of complex pathophysiology including neuroinflammatory processes and related reactive gliosis. Ketogenic diet (KD) is considered a solution in TBI due to its neuroprotective and anti-inflammatory properties.

AIM(S): The aim of this project was to evaluate the effects of KD on astrogliosis and microgliosis, and the levels of inflammatory markers after TBI.

METHOD(S): Ketogenic or standard diet (SD) were introduced on postnatal day 27 (P27) in male and female rats. Penetrating cortical brain injury was induced on P30. Animals were perfused 2, 8, 16, and 30 days post-injury (DPI). Brains were stained against glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1). GFAP-positive and Iba1-positive area fractions were quantified in perilesional cortex of injured animals and controls. The fractal and Sholl analysis of astrocytes and microglia in perilesional cortex was performed in FIJI. The other cohort of animals undergoing KD or SD was sacrificed 6h, 2 days or 30 days after brain injury, the cortical tissue was collected and homogenized and the levels of inflammatory markers were analysed using membrane-based antibody arrays.

RESULTS: KD did not alter GFAP-positive area fraction in perilesional cortex, but modified astrocyte morphology, reducing cell area, convex hull area, sum of intersections and ramifications index in both males and females after TBI. Iba1-positive area fraction was reduced in KD-fed injured females at 30DPI compared with SD-fed injured females. KD reduced the expression of LIX (LPS-induced CXC chemokine) in KD-fed females 6h after TBI compared to SD-fed injured females, with similar trend for MCP-1 (Monocyte chemoattractant protein 1).

CONCLUSIONS: Overall, ketogenic diet attenuated astrocyte hypertrophy in both sexes, modulated microgliosis and limited the expression of inflammatory factors in females following TBI.

FINANCIAL SUPPORT: National Science Centre Preludium 21 Grant (2022/45/N/NZ4/03028).

## S2P36. PRO-INFLAMMATORY AND PROLIFERATIVE EFFECTS OF NANOPLASTICS AND LIPOPOLYSACCHARIDE IN HUMAN BRAIN ENDOTHELIAL (HBEC-5I) AND ASTROCYTE CULTURES: IMPLICATIONS FOR NEUROVASCULAR DYSFUNCTION

Emilia Grzęda, Natalia Leciejewska, Agnieszka Kaczmarek, Piotr Grela, Ewa Pruszyńska-Oszmałek, Maciej Sassek, Paweł Antonii Kołodziejski

*Department of Animal Physiology and Biochemistry, Poznań University of Life Sciences, Poznań, Poland*

INTRODUCTION: Nanoplastics (NPs) are emerging contaminants that can cross biological barriers and accumulate in the CNS. Increasing evidence indicates that BBB dysfunction and neuroinflammation are early events in neurodegenerative disorders. However, the combined impact of NPs and inflammatory stimuli on human neurovascular cells remains insufficiently characterized.

AIM(S): We hypothesized that NPs potentiate LPS-induced inflammatory responses in human brain endothelial (HBEC-5i) and astroglial cells, thereby promoting neurovascular dysfunction.

METHOD(S): HBEC-5i and astrocyte cells were exposed to NPs, LPS, or a combination. Cell metabolic activity and viability were assessed using MTT and NRU assays, proliferative capacity was evaluated by BrdU test. Quantitative PCR was performed to assess selected inflammatory markers. All experiments were conducted under controlled in vitro conditions with appropriate controls.

RESULTS: Preliminary analyses indicate that exposure to NP+LPS induced more pronounced alterations in cellular metabolic activity and proliferation than single treatments. Notably, MTT and NRU assays showed an altered metabolic response under co-exposure conditions. BrdU incorporation assays demonstrated treatment-dependent modulation of proliferative activity. Gene expression analysis revealed treatment-dependent changes in pro-inflammatory markers, most pronounced in the NP+LPS group.

CONCLUSIONS: Our findings suggest that NPs may amplify inflammation-related responses in both cell types, particularly under conditions of concomitant immune activation. We observed additive effects of NP and LPS exposure, supporting the concept that NPs could exacerbate neuroinflammatory.

FINANCIAL SUPPORT: The project is financed from the state budget, allocated by the Minister of Science and Higher Education under the "Student science clubs create innovations" program.

## S2P37. SIGMA-1 RECEPTOR MODULATION AS A THERAPEUTIC STRATEGY FOR ENHANCING OLIGODENDROCYTE DIFFERENTIATION AND REMYELINATION

Kinga Tylek[1], Tommaso Fabiano[1], Giuseppe Romeo[2], Giampiero Leanza[2], Antonella Ragnini-Wilson[1]

[1] *Department of Biology, University of Rome "Tor Vergata", Rome, Italy*

[2] *Department of Drug and Health Sciences, University of Catania, Catania, Italy*

INTRODUCTION: Among the defining features of many central nervous system (CNS) diseases is demyelination, most prominently observed in Multiple sclerosis (MS), a chronic autoimmune and inflammatory disorder and the leading cause of disability in young adults. MS is characterized by focal demyelinating lesions in white matter of the brain and spinal cord, leading to progressive neurological impairment. Because endogenous remyelination is limited, the sigma-1 receptor (S1R) has gained attention as a therapeutic target. S1R acts as an intracellular chaperone regulating $Ca^{2+}$ homeostasis and ER-mitochondria signaling, reducing oxidative stress and mitochondrial dysfunction. Thus, S1R activation represents a mechanistic strategy to enhance oligodendrocyte lineage progression, promote remyelination, and improve white matter integrity.

AIM(S): We evaluated the promyelinating potential of S1R agonists in vitro using the oligodendroglial Oli-neuM model, which differentiates into a mature phenotype capable of remyelinating axons in vitro.

METHOD(S): Dose-dependent effects of two novel bifunctional S1R agonists (CVPO1 and GR01) on myelination were assessed by MBP immunofluorescence. Selected doses were then analyzed by qRT-PCR to evaluate effects on oligodendrocyte differentiation (Sox10, Gpr17), late myelin genes (Mog, Opalin), and ER stress markers (Atf4, Chop).

RESULTS: Our results showed increased Sox10 and myelination markers with reduced Gpr17 after CVPO1 and GR01 treatment, indicating enhanced oligodendrocyte differentiation. Importantly, both compounds reduce ER stress via different mechanisms: CVPO1 attenuates Chop levels, while GR01 decreases Atf4 gene expression.

CONCLUSIONS: Based on the presented results, it is possible to suggest that pharmacological targeting enhancement of the S1R activity may represent a promising therapeutic strategy for central nervous system demyelination disorders, including multiple sclerosis, by through the promotion of remyelination once lost as a result of pathology.

FINANCIAL SUPPORT: Supported by grant no. PRIN2022 – Prot. 2022ZSC4Z5.

## S2P38. DETERMINATION OF THE MOLECULAR MECHANISMS OF THE NEUROPROTECTIVE EFFECTS OF CBD AND CBG ON HUMAN HMC3 MICROGLIAL CELLS

Alicja Kisielewicz, Natalia Małek-Chudzik

*Department of Chemical Biology and Bioimaging, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland*

INTRODUCTION: Microglial cells are the central nervous system's resident immune cells, responding to disturbances like aggregated proteins or ATP. Their activation can trigger chronic neuroinflammation, accelerating neurodegeneration in disorders such as Alzheimer's and Parkinson's. Targeting this process is critical, yet preventive medications are lacking, highlighting the need for new therapies.

AIM(S): Cannabidiol (CBD) and cannabigerol (CBG) have shown antioxidant and anti-inflammatory properties. This study aimed to investigate their impact, alone and in combination, on ATP-stimulated human microglial cells (HMC3) to determine their potential to mitigate neuroinflammation.

METHOD(S): Our research was based on the analysis of cell proliferation and the cytotoxicity of compounds, performed by Incucyte Live-Cell Imaging System. The study evaluated microglial markers associated with pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes, alongside apoptotic markers, to identify molecular targets modulated by CBD and CBG. The analysis included markers such as iNOS, IL-1β, SOD, NOX-4, TNF-α, and FAAH.

RESULTS: We observed a dose- and time-dependent effect of the tested compounds on the number of PI-positive dead cells. The number of dead cells increased over time in all conditions, but higher concentrations of the compounds resulted in a smaller increase compared to the control. This indicates a dose- and time-dependent cytoprotective effect. For our studies, selecting concentrations that maintain cell viability while ensuring activation and moderate expression of inflammatory factors was critical for obtaining biologically relevant results. The analysis of inflammatory marker expression confirmed that both CBD and CBG significantly modulate these pathways.

CONCLUSIONS: CBD and CBG effectively shift activated microglia toward a homeostatic, neuroprotective phenotype. These findings support their therapeutic potential in managing neuroinflammation.

FINANCIAL SUPPORT: This work was supported by National Science Center grant 2023/49/B/NZ7/02172.

## S2P39. HOW DO VARIOUS COMPONENTS OF THE ENRICHED ENVIRONMENT AFFECT TRANSCRIPTOMIC RESPONSES IN MICROGLIA?

Kinga Szydlowska[1], Maciej Florczyk[1], Renan Tivanello[1], Zuzanna Luczak-Sobotkowska[1], Jan Brozek[1], Anthony J. Hannan[3], Rosa Chiara Paolicelli[4], Ali Jawaid[2], Bożena Kaminska[1]

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

[2] *Translational Neuropsychiatry Research Group, Lukasiewicz Research Network – PORT Polish Center of Technology Development, Wrocław, Poland*

[3] *Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Australia*

[4] *Department of Biomedical Sciences, University of Lausanne, Switzerland*

INTRODUCTION: Environmental enrichment (EE) is an experimental paradigm that combines physical, sensory, and cognitive stimulation and shows beneficial effects in pre-clinical models of neurodegeneration. Microglia are resident myeloid cells of the central nervous system (CNS), which, through bidirectional communication with other brain cells, maintain CNS homeostasis. In previous studies, EE partially reversed the pro-inflammatory effects of aging on microglia and attenuated the inflammatory response of microglia to Aβ oligomers, thereby promoting their cytoprotective and phagocytic phenotypes.

AIM(S): In this study, we investigated the transcriptomic responses of microglia to EE or its individual components.

METHOD(S): Mice (male and female) were subjected to the full EE or EE without physical exercise, EE without cognitive stimulation, EE without social engagement (single housing of mice); naïve mice or mice single housed without EE were used as controls. Microglia from hippocampi were immunosorted as CD11b+ cells by flow cytometry and subjected to RNA sequencing and computational analyses.

RESULTS: Principal component analysis did not show significant transcriptomic differences between groups in females; however, distinct transcriptomic patterns have been detected between various groups in males. We identified the gene signature associated with EE, which was, to a large extent, induced by physical activity. Gene Ontology (GO) analyses revealed various processes induced by specific EE components, distinct from those of a full EE.

CONCLUSIONS: Interestingly, stress caused by solitary housing abrogated EE-induced gene expression in microglia. Our results demonstrate specific microglial responses to a full EE protocol, as well as its components, shedding light on which elements enhance the protective and modulatory role of microglia.

FINANCIAL SUPPORT: tudies were supported by the National Science Center JPND 2022/04/Y/NZ5/00122.

## S2P40. DOES THE SORLA SORTING RECEPTOR MODULATE MICROGLIAL RESPONSE TO INTERFERON-Γ?

Klaudia Golis, Paulina Kamińska, Aleksandra Tempes, Aleksandra Klachacz, Tomasz Obrębski, Anna Malik

*Cellular Neurobiology Research Group, Faculty of Biology, University of Warsaw, Warsaw, Poland*

INTRODUCTION: Glioblastoma (GBM) reprograms glioma-associated microglia/macrophages (GAMs) toward tumor-supporting states. SorLA is an intracellular sorting receptor that limits microglial inflammatory activity, yet its role in immune signaling remains unclear. Based on our proteomics data from wild-type (WT) and SorLA-deficient (SorLA-KO) microglia co-cultured with glioma cells, we hypothesise that SorLA regulates interferon-γ (IFNγ) responses in brain myeloid cells.

METHOD(S): We used RT-qPCR to analyze expression levels of interferon-regulated genes in sorted CD11b$^+$ cells from gliomas in WT and SorLA-deficient mice. To assess the impact of SorLA loss on IFNγ signaling, we generated a SorLA-KO RAW264.7 macrophage line using CRISPR/Cas9. We used this newly established cell line and primary microglia cultures to investigate cellular responses to IFNγ, quantifying STAT1 phosphorylation and its nuclear translocation, and the expression of IFNγ target genes.

RESULTS: SorLA deficiency altered the proteome of primary microglia co-cultured with glioma cells. SorLA-KO microglia was characterized by higher abundance of interferon-induced and phagocytosis-related proteins. Further studies confirmed that SorLA-KO microglia showed increased phagocytosis. RT-qPCR of sorted CD11b$^+$ cells revealed a pro-inflammatory shift of SorLA-KO cells, with reduced expression of the markers of tumor-supportive phenotype (CD163, CD206, Arg1) and increased interferon-inducible genes (IFIT1, IFIT2). Currently we are investigating responses to both genotypes to IFNγ in cellular models.

CONCLUSIONS: Together, our data suggest that SorLA may act as a modulator of IFNγ-dependent signaling in microglia/macrophages, linking intracellular sorting to neuroinflammatory responses. This mechanism may have relevance for shaping the properties of the brain microenvironment in glioblastoma.

FINANCIAL SUPPORT: This research was supported by the NCN OPUS research grant 2020/37/B/NZ3/00761.

## S2P41. COMPARATIVE ANALYSIS OF CHEMOTHERAPY-INDUCED NEUROTOXICITY IN PRIMARY MOUSE NEURONAL CULTURES

Bernadeta A. Pietrzak-Wawrzyńska[1], Agnieszka Wnuk[1], Karolina Przepiórska-Drońska[2], Andrzej Łach[1], Wiktoria Płonka[1]

[1] *Team III, Department of Pharmacokinetics and Drug Metabolism, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Department of Experimental Neuroendocrinology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Chemotherapy-related cognitive impairment ("chemobrain") is a complex neurological consequence of anticancer treatment, the underlying mechanisms of which remain incompletely understood. Although chemotherapeutic agents are effective in eliminating cancer cells, they frequently also damage healthy brain cells, leading to long-lasting neurological dysfunction. Memory deficits affect over 90% of oncology patients and may persist for up to five years after cessation of treatment.

AIM(S): Despite the clinical relevance of this phenomenon, the existing literature lacks comprehensive comparative analyses that consistently evaluate neurotoxicity, dose-response relationships, and inter-agent differences. To address this gap, the aim of the present study was to directly compare the dose-dependent neurotoxic effects of commonly used chemotherapeutic agents from different pharmacological classes within a unified cell model.

METHOD(S): In this study, neuronal cells were treated with increasing doses of selected chemotherapeutic agents, including 5-fluorouracil, oxaliplatin, methotrexate, and paclitaxel. Neurotoxicity was assessed using biochemical assays. Primary cultures of mouse neuronal cells were employed, as the anti-proliferative nature of chemotherapeutic agents limits the suitability of immortalized cell lines for addressing this research question.

RESULTS: The results revealed pronounced time- and dose-dependent neurotoxicity across the tested chemotherapeutic agents from different pharmacological classes. The analysis encompassed their impact on neuronal cell viability, metabolic activity, and markers of cellular damage.

CONCLUSIONS: Collectively, these findings provide a solid foundation for further investigation into the mechanisms underlying chemotherapy-induced neurotoxicity and for the development of pharmacological strategies aimed at mitigating these adverse effects.

FINANCIAL SUPPORT: NCN Preludium 22 Grant no. 2023/49/N/NZ7/03640.

## S2P42. THE ROLE OF EXERCISE MIMETICS IN MODULATING THE PROANGIOGENIC PROPERTIES OF PRO-TUMORIGENIC NEUTROPHILS

Weronika Jurczyk[1], Mateusz Smolarz[1,2], Marta Nowacka-Chmielewska[1]

[1] *Institute of Physiotherapy and Health Sciences, Academy of Physical Education, Katowice, Poland*

[2] *Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice, Poland*

INTRODUCTION: Neutrophils constitute 50-70% of WBCs and play a significant role in modulating the tumor microenvironment. They exist in two phenotypes: anti- and pro-tumor. An important regulator of their function is the type 1 interferon receptor. Silencing this receptor promotes neutrophils to a pro-tumor phenotype (Ifnar1-/-). They can promote angiogenesis and modulate the tumor microenvironment by secreting mediators (VEGF, MMP-9). Current research indicates that small extracellular vesicles (sEVs) secreted by neutrophils may play a significant role in this modulation. Studies of exercise mimetics, including metformin and betaine, indicate that they may have anti-angiogenic and anti-tumor effects.

AIM(S): The aim of this study is to assess the potential effect of metformin and betaine on the pro-angiogenic properties of sEVs secreted by Ifnar-/- neutrophils.

METHOD(S): The study used ER-Hoxb8 progenitor cells with type 1 interferon knockout (Ifnar1-/-). To differentiate the cells into neutrophils, β-estradiol was removed from the medium 5 days prior to isolation. 48 hours before isolation the medium was changed to one with FBS depleted in exo. Isolation was performed using mini-SEC. During the differentiation period, the cells were stimulated with appropriate doses of metformin and resveratrol. Mimetics concentrations were determined based on the results of the MTT assay. The obtained sEVs were subjected to ELISA, VEGF, and MMP-9 assays.

RESULTS: The MTT assay revealed dose-dependent changes in cell viability. Based on this, optimal concentrations were selected for further testing. ELISA assays revealed a differential VEGF and MMP-9 profile in sEVs from Ifnar1-/- neutrophils treated with selected mimetics.

CONCLUSIONS: Our results suggest that mimicking the metabolic effects of physical activity using metformin and betaine may lead to changes in the pro-angiogenic profile of sEVs secreted by Ifna1r-/- neutrophils. This indicates a potential role of exercise-induced mechanisms in inhibiting tumorigenesis.

FINANCIAL SUPPORT: This research was funded by the NCN project: 2023/48/C/NZ5/00001.

## S2P43. DONOR-RELATED VARIABILITY IN EV SECRETION BY WJ-MSCS AND ASCS – CHALLENGES FOR PERSONALIZED CELL-FREE THERAPIES

Monika Rytel[1], Sylwia Katarzyna Król[2], Anna Sarnowska[1]

[1] *Translational Platform for Regenerative Medicine, Mossakowski Medical Research Institute, Warszawa, Poland*

[2] *Department of Neurooncology, Mossakowski Medical Research Institute, Warszawa, Poland*

INTRODUCTION: Mesenchymal stem/stromal cells possess immunomodulatory, regenerative, and neuroprotective properties, making them promising candidates for the treatment of neurological disorders. Their therapeutic effects largely depend on their secretome, which includes soluble factors and extracellular vesicles (EVs). EVs are key mediators of intercellular communication, carrying proteins, lipids, nucleic acids, and membrane receptors that can be transferred to recipient cells. Their ability to deliver functional biomolecules has generated strong interest in their use as biomarkers and therapeutic agents.

AIM(S): We hypothesized that MSCs derived from different sources secrete varying numbers of EVs and may therefore differ in their therapeutic potential. Thus, we compared two types of MSCs – WJ-MSCs and ASCs.

METHOD(S): Human umbilical cords (n=5) were obtained from full-term deliveries and MSCs were then mechanically isolated from Wharton's jelly (WJ-MSCs). Adipose tissue-derived MSCs (ASCs) were isolated enzymatically with collagenase from human adipose tissue (n=5). Next, both WJ-MSCs and ASCs were cultured until 3rd passage, at 5% O2, 5% CO2, 37°C. EVs were then isolated from cell culture medium by ultracentrifugation (300xg for 10 min. 4°C, 2 000xg for 30 min. 4°C, and 100 000xg for 1h 30 min. 4°C) The obtained EVs were suspended in PBS and their number and size was determined by NTA (Nanoparticle Tracking Analysis). EV morphology was analyzed by TEM (Transmission Electron Microscopy).

RESULTS: The analysis revealed that WJ-MSCs and ASCs obtained from different donors secrete different numbers of EVs. EVs isolated from both cell types represented a typical extracellular vesicle-like morphology according to TEM analyses.

CONCLUSIONS: WJ-MSCs and ASCs derived from different donors exhibit donor-dependent variability in the number of secreted EVs, which in the future may potentially have an impact on the efficiency of cell-free therapies dedicated for individual patients.

FINANCIAL SUPPORT: This research was funded by National Science Centre grant number 2022/47/O/NZ3/01739.

## S2P44. BIOLUMINESCENCE AND MRI ASSESSMENT OF GLIOBLASTOMA XENOGRAFT MODELS

Justyna Grymuza[1,2], Anna Niedziałek[3], Radosław Rola[2], Agata Chudzik[2]

[1] *Doctoral School of the Medical University of Lublin*

[2] *Department of Neurosurgery and Paediatric Neurosurgery, Medical University of Lublin, Lublin, Poland*

[3] *Department of Radiography, Medical University of Lublin, Lublin, Poland*

INTRODUCTION: Glioblastoma (GBM) is the most aggressive primary diffuse astrocytic glioma of the central nervous system (CNS). It is classified by the World Health Organization (WHO) as a grade 4 glioma and accounts for approximately 50% of malignant CNS tumors. Standard diagnosis relies on magnetic resonance imaging (MRI), which enables evaluation of tumor location and volume. Despite ongoing advances in surgical and chemoradiotherapeutic treatments, precise methods for assessing tumor progression, particularly in translationally relevant preclinical models, are needed.

AIM(S): The primary aim of this study was to establish a murine orthotopic GBM xenograft model using U-251MG-Luc cells stably transduced with luciferase to enable bioluminescence imaging (BLI) in the presence of luciferin as a substrate.

METHOD(S): The study employed a combination of BLI, which offers high sensitivity for detecting viable tumor cells, and MRI, which provides high spatial resolution. The integration of both methods enabled comprehensive weekly assessment of tumor engraftment, growth dynamics, and morphological progression up to

day 56. Additionally, an orthotopic U-87MG GBM xenograft model was developed as a comparative reference.

RESULTS: The results demonstrate that U-251MG-Luc cells formed tumors detectable by BLI earlier than by MRI, highlighting the higher sensitivity of the former. Both tested cell concentrations ($5 \times 10^5$ and $1 \times 10^6$) generated orthotopic xenografts with comparable growth and characteristics. U-87MG cells exhibited rapid growth detectable by MRI, allowing clear tumor visualization after 7 days. This model was successfully established and served as a reliable reference.

CONCLUSIONS: These findings highlight the utility of murine orthotopic xenograft models in GBM research and underscore the importance of combining imaging techniques in preclinical studies. Moreover, the study demonstrates the complementary nature of BLI and MRI in studies utilizing preclinical GBM models.

FINANCIAL SUPPORT: This study was supported by an internal grant for young researchers of the Medical University of Lublin.

## S2P45. IMMUNOTHERAPY OF GLIOBLASTOMA – THERAPEUTIC POTENTIAL OF ELIMINATING TUMOR-ASSOCIATED MACROPHAGES

Olga Malik[1,2], Natalia Mikołajczuk[1,2], Małgorzata Bajor[1], Magdalena Winiarska[1], Julia Maroszek[3,4], Christopher Forcados[5,6], Sébastien Wälchli[5], Maciej Szydłowski[3], Marta Kłopotowska[1]

[1] *Department of Immunology, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warszawa, Poland*
[2] *Immunooncology Students' Science Association, Medical University of Warsaw, Warszawa, Poland*
[3] *Department of Experimental Hematology, Institute of Hematology and Transfusion Medicine, Warszawa, Poland*
[4] *Doctoral School, Centre of Postgraduate Medical Education, Warszawa, Poland*
[5] *Translational Research Unit, Section of Cellular Therapy, Department of Oncology, Oslo University Hospital, Oslo, Norway*
[6] *Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo*

INTRODUCTION: Immunotherapies in treatment of Glioblastoma (GBM) are an attractive strategy, but they face numerous challenges, including highly immunosuppressive tumour microenvironment (TME). Among the many factors in the TME, tumour-associated macrophages (TAM) play a key role in tumour progression and resistance to therapies. Targeting TAMs appears to be an attractive approach to improve therapeutic strategies in GBM. In our studies, we identified a marker specific for TAMs, representing a novel potential target for TAMs depletion. We designed CAR-T cell construct directed against it and evaluated its efficacy in eliminating macrophages in vitro.

AIM(S): Overall objective of the project is to verify therapeutic potential of eliminating TAMs in GBM with CAR T cell strategy.

METHOD(S): Macrophages were generated either from human monocytic cell line THP1 or from primary human monocytes isolated from buffy coats, and differentiated with PMA and M-CSF, respectively. Macrophages were polarized towards TAMs with IL-4, IL-10 or by coculture with GBM cells (U251, U87, DK-MG) in a transwell system or with conditioned medium collected from GBM cultures. Surface antigens and induction of CAR-T target protein on macrophages were evaluated with flow cytometry and Western blotting.

RESULTS: We established a research model to study the interaction between GBM, TAMs and immune cells, mimicking the natural TME. In this model we confirmed changes in macrophages towards M2-like phenotype with increased level of CD206 antigen after co-culture with supernatants from U87 cells. In this group, we also observed the induction of protein-of-interest recognized by our CAR-T cell.

CONCLUSIONS: We confirmed induction of mentioned CAR T cell target on macrophages conditioned with GBM line which is a rationale for testing the CAR-T therapy directed against this antigen. We aim to expand our research model to test patient derived GBM cell lines exhibiting a stem cell phenotype and evaluate a panel of CAR constructs for their anti-TAM effectiveness.

FINANCIAL SUPPORT: The research was conducted as part of a grant Opus NCN 2022/45/B/NZ6/02588.

## S2P46. CHARACTERIZATION OF NOVEL CLUSTERS OF GLIOBLASTOMA PATIENTS WITH EGFR MUTATION

Clara E. Gavira-O'Neill[1,2], Juan Manuel Pastor[2], Carmen Ramírez Castillejo[2], Sergio Casas Tintó[1]

[1] *Institute of Rare Diseases Research, Human Disease Models in Drosophila Unit, Carlos III Health Institute, Majadahonda, Spain*

[2] *Higher Technical School of Agricultural, Food and Biosystems Engineering, Technical University of Madrid, Madrid, Spain*

INTRODUCTION: Glioblastomas (GB) are the most malignant primary brain tumors. Median survival is estimated at 15 months, although there is great heterogeneity amongst patients. To classify these phenotypically diverse cases there has been extensive research into the molecular background of GB patients, including identification of frequent mutations. For example, over 50% of GB cases present alterations in expression of epidermal growth factor receptor (EGFR), with over-expression associated to more aggressive GB. Multiple mechanisms lead to EGFR pathway activation but existence of different variants, and incomplete understanding of affected signalling pathways, have limited the application of treatments targeting this protein.

AIM(S): The presented project aims to specifically cluster these EGFR-altered patients and further characterize the biological pathways that differentiate groups.

METHOD(S): The algorithm applies unsupervised machine learning methods to GB patients' clinical and transcriptomic data for patient clustering, while further clarification of the biological basis of differences is done using a Drosophila melanogaster GB model.

RESULTS: Our algorithm identifies subgroups with differential patterns in tumor-associated biological processes, including significant differences in stemness, cell-cell communication, inflammation, chromatin destabilization, and tumor expansion. Identified genes of interest are also associated to differences in survival in the Drosophila model.

CONCLUSIONS: Further characterization of these stratifying mechanisms can lead to better understanding of tumor progression and the differences in response to treatments, with a final goal of more personalized tumor management.

FINANCIAL SUPPORT: The project is funded by a FPI project from the Comunidad de Madrid – IND2023/BMD-28759.

## S2P47. AN *IN VIVO* FAST NEUROIMAGING APPROACH TO MODEL PATIENT SURVIVAL IN HUMAN GLIOBLASTOMA

Anna Janus[1,2,9], Joan Falcó Roget[2], Julia Rybska[2], Barbara Wojtarowicz[2], Claudio di Paola[3], Augusto Lelo[4], Sara Lillo[5,6], Letterio Salvatore Politi[7,8], Jan K. Argasiński[2,8], Alberto Cacciola[7,8]

[1] *Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*

[2] *Computational Neuroscience Group, Sano Centre for Computational Medicine, Kraków, Poland*

[3] *Department of Biomedical, Dental Sciences and Morphological and Functional Imaging, University of Messina, Messina, Italy*

[4] *Neurolesi Center, Scientific Institute for Research, Hospitalization and Healthcare, Messina, Italy*

[5] *Radiation Oncology Unit, Clinical Department National Center for Oncological Hadrontherapy, Pavia, Italy*

[6] *Department of Internal Medicine and Medical Therapy, University of Pavia, Pavia, Italy*

[7] *Department of Biomedical Sciences, Humanitas University, Milan, Italy*

[8] *Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Kraków, Poland*

[9] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Glioblastoma (GBM), the most malignant primary brain tumour, exists within the context of neural circuits. White matter tracts constitute major routes of tumour invasion and progression. Additionally, GBMs engage in bidirectional mechanisms of neuron-cancer communication, altering the topology of the brain connectome while exploiting functional synapses to grow and recur.

AIM(S): We aimed to develop a circuitry-oriented framework that may enhance our understanding of GBM evolution and ultimately improve survival prediction and disease prognosis.

METHOD(S): We used a normative framework in four independent cohorts of patients diagnosed with GBM according to the WHO 2021 criteria (N=367, 496, 103, and 33). For each patient, multimodal MRI and tumor segmentation masks were normalized to a common MNI template. Then, we extracted the subset of streamlines from a normative tractogram that intersected the lesion masks and obtained patient-specific maps of GBM-brain structural scaffolds. We computed the corresponding tract density and network indices. Lastly, we combined these with survival analysis using Cox and Kaplan-Meier curves to study the association between the circuitry of GBM and patient outcomes.

RESULTS: Low-dimensional embeddings of white matter density maps identified macroscale patterns of white matter involvement. These components signifi-

cantly improved survival risk prediction compared to clinical covariates alone (C-index: 0.72 *vs.* 0.66). Network metrics showed significant univariate associations with survival in selected cases ($p<0.001$; C-index ≥0.56), while others were non-informative. Joint representations uncovered unique topological profiles associated with distinct survival trajectories (Kendall's $\tau$, $p<0.05$).

CONCLUSIONS: We present a robust approach to characterize the macroscale structural interactions between GBMs and the human connectome to define a topological risk score and stratify patients to enhance disease prognosis.

FINANCIAL SUPPORT: EU Horizon 2020 (857533), FNP (MAB PLUS/2019/13), MEiN (MEiN/2023/DIR/3796).

## S2P48. LOOKING BEYOND THE OPERATIVE FIELD: REMOTE CEREBELLAR HAEMORRHAGE (RCH) AFTER SUPRATENTORIAL ANEURYSM CLIPPING

Iga Łobińska[1], Michał Krakowiak[2]

[1] *Student's Scientific Circle of Neurosurgery, Medical University of Gdańsk, Gdańsk, Poland*

[2] *Department of Neurosurgery, Medical University of Gdańsk, Gdańsk, Poland*

INTRODUCTION: Remote cerebellar haemorrhage (RCH) is an uncommon, infratentorial complication with a reported incidence ranging from 0.04% to 0.8%. This condition can develop after various neurosurgical procedures, mostly following supratentorial craniotomies. Typically identified on computed tomography (CT) by the characteristic "zebra sign." Owing to nonspecific clinical presentation RCH remains an underdiagnosed postoperative complication and should be considered in the differential diagnosis of neurological deterioration after cranial surgery.

AIM(S): To illustrate a rare but clinically significant complication of supratentorial aneurysm surgery and emphasize its diagnostic implications.

METHOD(S): A single-patient case analysis was performed. A 65-year-old male undergoing elective supratentorial aneurysm clipping was assessed postoperatively using serial neurological examinations and computed tomography imaging. The diagnosis of RCH was established based on the characteristic distribution of infratentorial bleeding and temporal relationship to surgery.

RESULTS: Conservative management was pursued. Serial imaging demonstrated radiological stabilization with gradual regression of haemorrhagic lesions despite transient cerebrospinal fluid leakage. Recurrent seizures complicated the clinical course. The patient was discharged after prolonged hospitalization with residual left lower limb paresis and cerebellar syndrome.

CONCLUSIONS: The pathophysiology of RCH remains incompletely understood. Current evidence suggests that abrupt alterations in intracranial pressure, cerebrospinal fluid loss, arterial hypertension, perioperative seizures play a pivotal role. This case underscores the importance of recognizing RCH as a potential postoperative complication and demonstrates that, despite significant morbidity, conservative management may result in a favorable neurological outcome.

FINANCIAL SUPPORT: None.

## S2P49. IMPLEMENTATION OF IN-HOUSE CELL-BASED ASSAYS FOR THE DETECTION OF NEURAL AUTOANTIBODIES IN CEREBROSPINAL FLUID

Olgerd Babkin[1,2], Neringa Daugelaviciene[1,2], Mantas Vaisvilas[3], Urtė Neniškytė[1,2]

[1] *Institute of Bioscience, Life Sciences Center, Vilnius University, Vilnius, Lithuania*

[2] *VU LSC-EMBL Partnership for Genome Editing Technologies, Life Sciences Center, Vilnius University, Vilnius, Lithuania*

[3] *Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania*

INTRODUCTION: The identification of disease-specific neural antibodies has paved the way for a new subspecialty in clinical medicine known as autoimmune neurology.

AIM(S): This is an exciting field for clinical and academic work because accurate diagnosis through the identification of neural antibodies can lead to complete remission of severe neurological impairment associated with these conditions. However, this clinical promise hinges on the precision of the diagnostic platform.

METHOD(S): Unlike traditional solid-phase assays, the Cell-Based Assay (CBA) platform expresses antigens in their native, three-dimensional conformational states within eukaryotic cells – a methodological approach critical for maintaining diagnostic sensitivity and specificity.

RESULTS: In this study, we successfully implemented an in-house CBA to detect autoantibodies against glial fibrillary acidic protein (GFAP) and cerebellar degeneration-related protein 2-like (CDR2L) in patient cerebrospinal fluid.

CONCLUSIONS: Integrating these specialized assays into diagnostic workflows has the potential to enhance detection sensitivity and provide the high-fidelity data necessary for more accurate diagnosis and targeted treatment strategies.

FINANCIAL SUPPORT: Adenovirus vectors for clinical gene transfer HORIZON.3.1 – The European Innovation Council (EIC) Grant agreement ID: 101098647.

## S2P50. ENHANCEMENTS OF BLOOD-BRAIN BARRIER TRANSPORT ENABLED BY ADJUSTING THE SIZE OF NANOPARTICLE DRUG CARRIERS, AND SEARCHING FOR CONDITIONS TO PRODUCE THE MOST OPTIMAL CARRIER

Małgorzata Wojciechowska

*Faculty of Biology, Jagiellonian University, Kraków, Poland*

INTRODUCTION: The blood-brain barrier (BBB) severely limits effective delivery of pharmaceutics to the brain, which poses significant clinical trouble especially in situations where the time window for drug administration is short, ie. in case of a stroke. There arises a need to design drug carriers yielding the best BBB penetration rate. Taking into account the complexity of BBB, no uniform template can be invented, so instead the project focuses on the prospect of highly customisable nanoparticle drug carrier capsules, investigating the extent to which their size can be controlled.

AIM(S): 1. Investigate whether the duration of synthesis reaction has an impact on the size of produced nanoparticles (NP) 2. Investigate to what extent the diameter of produced NP can be controlled (range of values, standard deviation (SD)) 3. Find the conditions required for synthesis of optimal for BBB penetration NP size (~50nm, supported by scientific articles)

METHOD(S): Green synthesis of NP, samples taken at three times: after 30 minutes, 40 minutes, and 60 minutes. DLS and TEM scans to review PDNP diameter and variance in size within groups.

RESULTS: The variance, SD and mean of nanoparticle diameters increases with reaction time. 30min: mean- 24.40nm; variance-7.380; SD-2.717 40min: mean-26.99nm; variance-32.721; SD-5.720 60min: mean-52.60nm; variance-68.123; SD-5.720.

CONCLUSIONS: The diameter of NP can be controlled via adjusting reaction time, however the growth rate of NP becomes increasingly more erratic with time. Optimal for BBB transport NP were found in the 60min group, however the same group had the biggest variance and SD, suggesting decreased uniformity. While the 60min synthesis time yields the optimal NP size, it must be noted that there are many factors affecting the molecules ability to penetrate BBB, and depending on chosen method of transport, further modifications to NP structure must be made. Future extensions of this project should focus on applying cellular models to mimic in vitro efficiency.

FINANCIAL SUPPORT: None.

## S2P51. IMPROVING ENSEMBLE CLASSIFICATION OF STROKE USING A FEATURE SELECTION PIPELINE

Agata Leszczak[1,2], Jan K. Argasiński[2], Luca Gherardini[3], Cemal Koba[2]

[1] *Computational Neuroscience Team, Sano Centre for Computational Personalized Medicine, Kraków, Poland*

[2] *Jagiellonian University, Faculty of Biophysics, Biochemistry and Biotechnology, Kraków, Poland*

[3] *Computational Intelligence Team, Sano Centre for Computational Personalized Medicine, Kraków, Poland*

INTRODUCTION: Stroke is a neurological injury caused by disrupted cerebral blood flow. Neuroimaging technologies, such as fMRI, are fundamental for assessing post-stroke brain changes. They provide Functional Connectivity (FC) matrices that represent high-dimensional organization; nonetheless, their complexity challenges clinical interpretation and hinders fitting for Machine Learning (ML) models, especially when few samples are available.

AIM(S): We aim to provide a rigorous feature selection pipeline to enhance classification performance by preserving only the most relevant biomarkers.

METHOD(S): From fMRI data, we extracted whole-brain FC matrices (using Schaefer parcellation with 400 regions) for 31 controls and 154 stroke subjects. To address the high dimensionality of FC matrices, we developed a dual-stage feature selection pipeline comprising a Lasso regression and a Variance Inflation Factor to preserve independent and informa-

tive features. An ensemble of ML methods is exploited to perform collective classification in a case study on brain stroke. We considered the balanced accuracy achieved by the ensemble in predicting the presence of stroke using the full and filtered feature sets to evaluate the effect of the filtration.

RESULTS: Our feature selection pipeline substantially improved classification performance compared to the non-refined feature set. The best ensemble on the original dataset achieved a balanced accuracy of 59.95%, and the best on the filtered dataset achieved 74.6%, resulting in a relative increase in balanced accuracy of 24.44%. The feature selection pipeline we proposed has proven effective in increasing the balanced accuracy of the tested ensembles.

CONCLUSIONS: Future work will focus on testing the strength of the feature selection pipeline by adding multimodal data to the models, exploring its utility in predicting long-term clinical outcomes, and validating these biomarkers in larger and more diverse cohorts.

FINANCIAL SUPPORT: This project was funded by National Science Center, Poland, grant no 2024/55/D/NZ5/02998. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 857533 and from the International Research Agendas Programme of the Foundation for Polish Science No MAB PLUS/2019/13. The project was created within the project of the Minister of Science and Higher Education "Support for the activity of Centers of Excellence established in Poland under Horizon 2020" on the basis of the contract number MEiN/2023/DIR/3796. We acknowledge Polish high-performance computing infrastructure PLGrid (HPC Center: ACK Cyfronet AGH) for providing computer facilities and supportwithin computational grant no. PLG/2025/01828.

## S2P52. OCULOMOTOR VARIABILITY FROM RESTING-STATE FMRI AS A LONGITUDINAL MARKER OF STRUCTURAL BRAIN ALTERATIONS AFTER ISCHEMIC STROKE

Katarzyna Libera[1,2], Cemal Koba[1], Jan K. Argasiński[1]

[1] *Sano Centre for Computational Personalized Medicine, Kraków, Poland*

[2] *Faculty of Physics, University of Warsaw, Warszawa, Poland*

INTRODUCTION: Eye movement abnormalities are common after ischemic stroke, reflecting both localized damage and broader network disruptions. Resting-state fMRI-derived ocular variance (fMRIeye) tracks clinical severity and functional brain deviations after stroke, but its relationship to structural damage remains unclear.

AIM(S): We hypothesized that fMRIeye serves as a non-invasive marker of lesion volume, white-matter (WM) integrity, and longitudinal structural recovery, with differential effects for contralesional versus ipsilesional eyes.

METHOD(S): We analyzed 138 stroke patients scanned at 2 weeks, 3 months, and 1 year. Structural MRI included DWI, with lesion masks and diffusion metrics (ADC, FA). Ocular variance regressors were derived from eye regions of fMRI using PCA, separately for each eye. Linear mixed-effects models examined longitudinal associations between ocular variance, lesion metrics, and eye–lesion laterality.

RESULTS: fMRIeye was associated with lesion size and WM disconnection, particularly at 2 weeks. WM disease and lacunae were positively related to oculomotor variance bilaterally, with modest trends for stronger effects in the eye ipsilateral to the lesion. These associations persisted at 3 months and 1 year, indicating enduring effects of lesion burden on oculomotor stability. Lesion side, position, and type did not significantly explain variance, suggesting PCA variance primarily reflects global rather than focal structural damage. Microstructural metrics (ADC, FA) showed weak associations at later follow-ups.

CONCLUSIONS: Overall, fMRIeye provides a sensitive, non-invasive marker of structural brain alterations after stroke. It reflects global lesion burden and structural disconnection, tracks longitudinal changes in WM integrity, and highlights enduring effects of stroke injury on oculomotor stability. These findings support the utility of fMRIeye for clinical MRI datasets and underscore the prognostic value of oculomotor information in monitoring stroke progression and recovery.

FINANCIAL SUPPORT: This project was funded by National Science Center, Poland, grant no 2024/55/D/NZ5/02998. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 857533 and from the International Research Agendas Programme of the Foundation for Polish Science No MAB PLUS/2019/13. The project was created within the project of the Minister of Science and Higher Education "Support for the activity of Centers of Excellence established in Poland under Horizon 2020" on the basis of the contract number MEiN/2023/DIR/3796. We acknowledge Polish high-performance computing infrastructure PLGrid (HPC Center: ACK Cyfronet AGH) for providing computer facilities and supportwithin computational grant no. PLG/2025/01828.

## S2P53. HEARING IMPAIRMENT – INFLUENCE ON MRI-BASED BRAIN AGING MODEL ANALYSIS

Maria Waligórska[1,2,3], Gabriela Kępczyńska[3], Dominika Oziębło[3], Jarosław Żygierewicz[1], Tomasz Wolak[2]

*[1] Faculty of Physics, University of Warsaw, Warszawa, Poland*

*[2] Bioimaging Research Center, Institute of Physiology and Pathology of Hearing, Kajetany, Poland*

*[3] Department od Genetics, Institute of Physiology and Pathology of Hearing, Kajetany, Poland*

INTRODUCTION: Calculating the gap between estimated brain and chronological age may support early identification of age-related brain disorders and differentiation between patient groups. Our findings suggest that brain regions derived from MRI using FreeSurfer's ASEG segmentation and the A2009 atlas predict age accurately.

AIM(S): To explore machine learning models predicting brain age and assess deviations from the healthy aging trajectory across different hearing loss groups using brain morphometric features derived from the A2009 atlas and ASEG.

METHOD(S): Subjects were divided according to the BIAP hearing loss classification system based on tonal audiometry – 5 bilateral hearing groups, as well as a unilateral hearing loss group. For each brain feature, a polynomial function was calculated for the control group (separately for each sex). To account for the brain aging trend, a quadratic trend was included in the model. An OLS regression model was fitted with the scaled feature as a dependent variable and group status, age, and age$^2$ as independent variables.

RESULTS: The observed alterations included, among others, the bilateral planum temporale and the left suborbital sulcus, encompassing regions central to auditory and language processing as well as orbitofrontal networks involved in emotional and decision-related functions. Additionally, the left Heschl's gyrus within the superior temporal gyrus showed alterations, highlighting regions crucial for primary auditory processing. However, unilateral hearing loss groups seem to have smaller deviations, which may reflect the integration of inputs across left and right auditory pathways. Nevertheless, several factors should be taken into account when making such comparisons, including the age distribution of each group and the duration of hearing impairment.

CONCLUSIONS: Notably, profound hearing loss is often accompanied by decreased social interaction and cognitive stimulation, potentially contributing to reduced cognitive reserve.

## S2P55. CHARACTERIZATION OF BRAIN INJURY IN SUBJECTS WITH RADIOLOGICALLY ISOLATED SYNDROME SUSPECTED OF ASYMPTOMATIC MULTIPLE SCLEROSIS USING COGNITIVE FUNCTION AND BRAIN STRUCTURE VOLUME

Marta Agnieszczak, Maria Sygidus, Bartosz Kossowski, Maciej Juryńczyk

*Laboratory of Brain Imaging, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa, Poland*

INTRODUCTION: Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the CNS characterized by white matter lesions and diffuse neurodegeneration leading to visual, sensory, motor and cognitive dysfunction. Radiologically isolated syndrome (RIS) refers to asymptomatic individuals with MRI findings suggestive of MS. Some RIS individuals may fulfill the recently revised diagnostic criteria for MS based on cerebrospinal fluid findings but the extent of underlying damage and prognosis are largely unknown.

AIM(S): This study aimed to examine cognitive performance and brain structure volume in RIS subjects, patients with clinically definite MS, and healthy controls (HC).

METHOD(S): 29 RIS subjects, 27 MS patients and 22 HC underwent cognitive testing (SDMT, CVLT-II, BVMT-R, Digit Span, Stroop task) and 3T MRI. Cognitive impairment was defined as ≥2 scores below 1.5 SD. Brain volumes (whole brain, thalamus, hippocampus, cortical thickness) were assessed using FreeSurfer.

RESULTS: MS patients had lower normalized SDMT (-0.519 ± 0.778) when compared with RIS (-0.172 ± 0.827) and HC (0.265 ± 1,033, p=.008). No significant group differences were found in other cognitive domains. Cognitive impairment was identified in 4/29 RIS and 4/27 MS subjects. MS patients had lower thalamic volume (mean 8.074 ± 0.829, p<.05), when compared with RIS (8.552 ± 0.736) and HC (8.864 ± 0.710). In regression analyses, mean cortical thickness (p=.003) and thalamic volume (p=.015) predicted SDMT scores while thalamic volume predicted BVMT performance (p=.009).

CONCLUSIONS: As reported previously, MS patients showed impairment in information processing speed associated with lower thalamus volume. While RIS subjects on a group level did not differ significantly from HC in cognitive function or brain structure volume, 14% fulfilled the criteria for cognitive impairment, in-

dicating heterogeneity. Longitudinal analyses will determine the predictive value of cognitive and volumetric measures for conversion to clinically definite MS.

FINANCIAL SUPPORT: The study is funded and conducted as part of the research component of the National Science Centre 2021/42/E/NZ5/00213. "Predicting prognosis in asymptomatic individuals with multiple sclerosis-like brain lesions using cognitive tests and advanced magnetic resonance imaging techniques."

## S2P56. A CASE REPORT OF CHARCOT–MARIE–TOOTH DISEASE: CLINICAL PRESENTATION AND GENETIC ANALYSIS

Sandeep Thapa, Santosh khanal, Govardhan Joshi, Nilam Thakur

*Kathmandu Center for Genomics and Research Laboratory, Kathmandu, Nepal*

INTRODUCTION: Charcot-Marie-Tooth disease (CMT) represents a heterogeneous group of inherited peripheral neuropathies characterized by progressive motor and sensory dysfunction. It primarily affects the peripheral nerves, leading to distal muscle weakness, sensory loss, gait abnormalities, and skeletal deformities.

AIM(S): The aim of this study was to describe the clinical presentation, genetic findings, and diagnostic evaluation of a paediatric patient with Charcot-Marie-Tooth disease caused by a pathogenic ORC2 gene mutation, and to emphasize the importance of early molecular diagnosis in improving clinical management and prognosis.

METHOD(S): A 9-year-old female presenting with painful progressive myopathy, lower limb weakness, and recurrent falls was clinically evaluated. Detailed medical and family history were obtained, followed by comprehensive neurological examination. Laboratory investigations and neuroimaging were performed to exclude acquired neuromuscular conditions. Genomic DNA was extracted from peripheral blood, and comprehensive genetic analysis was conducted using next-generation sequencing. Variants were prioritized based on pathogenicity predictions, allele frequency in population databases, inheritance pattern, and correlation with the patient's phenotype. Functional relevance was assessed using published human and model organism data.

RESULTS: Genetic analysis identified a heterozygous pathogenic missense variant in the ORC2 gene: c.754C>T, resulting in the p.(Arg252Trp) amino acid substitution (rs864309503). This variant affects the ATPase domain of ORC2, which plays a critical role in chromatin modification, DNA repair, transcriptional regulation, and epigenetic silencing.

CONCLUSIONS: This case report highlights a rare pathogenic ORC2 gene mutation as the underlying cause of paediatric Charcot-Marie-Tooth disease, expanding the genetic landscape of inherited peripheral neuropathies.

## S2P57. PRESENTING TO THE ER: A CASE REPORT IN THE EMERGENCY DEPARTMENT

Wojciech S. Gaweł[1], Jacek Szypenbejl[1,2], Justyna M. Fercho[1,2,3], Oskar G. Chasles[1], Julia Nakoneczna[1], Zuzanna Krasula[1], Bogdan Jabłoński[1], Weronika Jagieło[1], Mariusz Siemiński[2]

[1] *Department of Emergency Medicine, Scientific Circle of Neurotraumatology, Medical University of Gdańsk, Gdańsk, Poland*

[2] *Department of Emergency Medicine, Medical University of Gdańsk, Gdańsk, Poland*

[3] *Department of Neurosurgery, 10th Military Research Hospital and PolyClinic SPZOZ, Bydgoszcz, Poland*

INTRODUCTION: Posterior Reversible Encephalopathy Syndrome (PRES) is a rare neuroradiological entity with heterogeneous triggers and variable lesion distribution. Acute seizures, altered consciousness, and visual symptoms may mimic stroke, infection, demyelination, or toxic–metabolic encephalopathy. The primary pathology of PRES is unknown, but the hallmark is cerebral edema. To date, no universal criteria for the diagnosis of PRES exist; however, a characteristic clinical presentation and the neuroradiological findings appear to be the most widely accepted.

AIM(S): We aim to present an atypical, severe PRES course in a complex patient and emphasize the role of early neuroimaging and broad differential diagnosis in the ER and ICU settings.

METHOD(S): The study is a retrospective case report. Clinical, laboratory, and imaging data were obtained from the patient's medical records.

RESULTS: A 35-year-old man with end-stage renal disease on dialysis, SLE, refractory hypertension, and ongoing immunosuppression was admitted after 5 days of unknown downtime with coma and suspected seizures. He presented in profound shock with severe metabolic derangements, coagulopathy, and extreme inflammatory markers. CT showed parieto-occipital white-matter hypodensities and edema suggestive of

PRES; MRI confirmed extensive lesions, while MRA/MRV excluded RCVS and venous thrombosis. Despite seizure prophylaxis, the prolonged ICU stay was complicated by polymicrobial/fungal infections, major hemorrhagic events, catheter thrombosis, and progression of cerebral edema with hemorrhagic transformation on follow-up imaging, leaving persistent neurological deficits.

CONCLUSIONS: PRES should be considered in critically ill patients with renal failure, autoimmune disease, immunosuppression, and sepsis, even when presentation is dominated by shock and metabolic abnormalities. Rapid CT followed by confirmatory MRI is pivotal to differentiate PRES from competing diagnoses and to guide management and prognostication.

FINANCIAL SUPPORT: There was no financial support provided.

## S2P58. REWIRING ACTION AND REWARD CONNECTION: RESTING-STATE FMRI CORRELATES OF CBT-INDUCED IMPROVEMENT IN ACADEMIC PROCRASTINATION

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

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

*[2] Poznań Laboratory of Affective Neuroscience, Institute of Psychology, SWPS University, Poznań, Poland*

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

INTRODUCTION: Procrastination is a problematic form of task delay associated with negative outcomes. Although cognitivebehavioral therapy (CBT) is considered one of the most effective interventions, the underlying psychological and neural mechanisms remain unknown.

AIM(S): The present randomized controlled trial addressed this gap by investigating changes in resting-state functional connectivity (RSFC) associated with CBT for procrastination.

METHOD(S): Highly procrastinating university students were randomized into a five-week online group CBT or a waiting-list control group. Participants (CBT=78, WL=35) completed 7-minute resting-state fMRI scans and questionnaire assessments before and after the intervention period. Behavioral change was measured with the Aitken Procrastination Inventory-Revised (API-R). Fourteen regions of interest implicated in emotion regulation, self-control, and episodic prospection were selected for seed-to-voxel analyses. Within the CBT group, regression models were applied to examine RSFC changes correlating with changes in API-R scores.

RESULTS: Behavioral improvement was significantly greater in the CBT group compared to controls (d=-0.68). Within the CBT group, seed-to-voxel analyses identified two patterns that significantly correlated with changes in API-R scores. The score improvement was associated with decreased negative connectivity (p-FDR=.006, β=-.015) between the medial orbitofrontal cortex (mOFC) and right precentral gyrus (PCG), and with decreased positive connectivity (p-FDR=.046, β=.017) within the right dorsolateral prefrontal cortex (dlPFC).

CONCLUSIONS: The shift of mOFC-PCG RSFC towards less negative values may reflect altered coactivation of action- and reward-related regions. Decreased local dlPFC connectivity could indicate greater functional flexibility of this key self-control region. Overall, the findings provide initial evidence for neural mechanisms underlying CBT for procrastination, highlighting the roles of reward processing and self-control.

FINANCIAL SUPPORT: This research was conducted by the GRAPPA consortium (General Research Assessing Psychotherapy for Procrastination Applications) and funded by the Polish National Science Centre grant 2021/43/B/HS6/02024 awarded to MW, JMM, and JK.

## S2P59. BODILY SELF-OTHER DISTINCTION RELATES TO HIGHER-ORDER SELF EXPERIENCES AND COGNITIVE SELF-OTHER DISTINCTION

A Enmalm[1,2], Paweł Orłowski[3], Thibault Scordel[1], Johanna Radegård[1], Rebecca Boehme[1,2]

[1] *Center for Social and Affective Neuroscience, Linköping University, Linköping, Sweden*

[2] *Center for Medical Image Science and Visualization, Linköping University, Linköping, Sweden*

[3] *Centre for Brain Research, Jagiellonian University, Kraków, Poland*

INTRODUCTION: The self is often conceptualized as hierarchical, where the bodily self is the basis for higher order self-experiences. An integral part of the self-experience is the ability to distinguish between the self and non-self across sensory experiences, actions and higher-order aspects. Touch is one of the most interesting ways to investigate the self-other distinction, as touch can be of significant social importance. Self-touch is a special case where we are both touching and touched, yet perceptual intensity is decreased. This reduced intensity shows the complex processes underlying the self-other distinction. There is also bidirectional influence of bodily and higher order processes, which is highlighted by how agency influences the tactile self-other distinction.

AIM(S): In the current work, we investigated how the bodily process of tactile self-other distinction relates to the higher order aspect of self-referential thinking and the self-concept.

METHOD(S): We used fMRI and behavioral measures to investigate the bodily process of tactile self-other distinction, and how it relates to the higher order aspect of self-referential thinking and the self-concept. We also investigated how they both relate to the sense of agency.

RESULTS: We found substantial overlap in neural processing of both self-other distinction and the higher-order self in brain areas involved in both self-processing such as the ACC, but also in social areas such as left inferior temporal gyrus. Further, other-self touch preferences related to the self-concept.

CONCLUSIONS: Our findings indicate shared neural substrates between the bodily and higher order processes of self and self-other distinction. We find the social component to be of particular importance in the self-experience, supporting the notion of a social bodily self.

FINANCIAL SUPPORT: The project was supported by Horizon Europe Grant 101115653 awarded to Rebecca Boehme.

## S2P60. LONGITUDINAL CHANGES IN PHONOLOGICAL PROCESSING IN THE LEFT VENTRAL OCCIPITOTEMPORAL CORTEX: FMRI EVIDENCE FROM TYPICAL READERS AND CHILDREN WITH DYSLEXIA

Agnieszka Mankiewicz[1], Agnieszka Dębska[2]

[1] *University of Warsaw, College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, Warsaw, Poland*

[2] *Laboratory of Language Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Reading acquisition is a complex process that relies on the systematic mapping of orthographic symbols onto phonological representations. It fundamentally reshapes phonological processing, leading to the automatic co-activation of orthographic information even during purely auditory tasks. This developmental pattern is disrupted in dyslexia, where degraded phonological processing results in failure to establish the integrated system.

AIM(S): This study investigated how the left ventral occipitotemporal cortex (lvOT) supports the development of orthography–phonology coupling in typical and atypical reading, focusing on the posterior–anterior gradient proposed to reflect a trajectory from small- to large-grain processing.

METHOD(S): We conducted a longitudinal fMRI study with 61 Polishspeaking children (20 with dyslexia, 41 controls). Participants were scanned twice: at the onset of formal reading instruction (TP1) and roughly three years later (TP2). Children completed three auditory phonological tasks probing different grain sizes: an alliteration task (smallgrain), a rhyme task (largegrain), and a wordmatching control. An orthographic visuallocalizer contrast delineated individual posterior and anterior lvOT regions of interest (ROIs). Activation within both ROIs was analysed using mixedeffects models.

RESULTS: ROI analyses revealed increased activation from TP1 to TP2 during the alliteration condition in both anterior and posterior lvOT (all $p \leq .01$). No longitudinal changes were observed for rhyme or control conditions, and no posterior–anterior shift in activation was found. This pattern was similar in both groups.

CONCLUSIONS: These findings indicate increasing lvOT involvement in small-grain phonological processing during reading acquisition, without evidence of posterior–anterior reorganisation. Comparable developmental trajectories across groups provide no evidence for delayed specialisation along the lvOT posterior–anterior axis in dyslexia, suggesting its core deficits may lie elsewhere.

FINANCIAL SUPPORT: This study was supported by the National Science Centre (Narodowe Centrum Nauki) grant number 2024/54/E/HS6/00242.

## S2P61. LEARNING BRAIN – A LONGITUDINAL DATASET ON NEURAL PLASTICITY DYNAMICS IN BRAILLE AND MUSIC TRAINING

Alicja M. Olszewska, Maciej Gaca, Dawid Droździel, Bartosz Kossowski, Aleksandra M. Herman, Artur Marchewka
*Laboratory of Brain Imaging, Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Human learning and adaptation rely on neuroplastic processes, the brain's intrinsic capacity to reorganise in response to experience. Understanding the temporal dynamics of these processes has far-reaching implications for neuroscience, psychology, or rehabilitative medicine.

AIM(S): To understand the dynamic nature of functional neuroplasticity using by multiple repeated measures and a comparative control group.

METHOD(S): The LEARNING BRAIN study investigated the neural mechanisms of experience-dependent plasticity resulting from different training paradigms: intensive musical training (N=24), tactile Braille learning (N=17), and a control group undergoing standard university curriculum (N=19). Over one year, the participants underwent domain-general and domain-specific functional magnetic resonance imaging (fMRI) tasks across 6–7 time points and behavioural assessments at 3 time points. This sampling paradigm enabled the characterisation of non-linear trajectories in neural adaptation.

RESULTS: Tactile Braille learners showed rapid recruitment of the inferior frontal gyrus for a Lexical Decision Task after just one week of training, whereas activation in the Visual Word Form Area (VWFA) emerged after six weeks. In the musical training group, the left and right sensorimotor cortices showed parallel changes for a piano playing task within the first three months of training, and diverged afterwards.

CONCLUSIONS: These results show distinct, dynamic training- and task-specific patterns of brain reorganisation. To facilitate further discovery, we have released the complete preprocessed fMRI and behavioural dataset, including 6 fMRI tasks and 2 cognitive behavioural tasks, via OpenNeuro (https://openneuro.org/datasets/ds007022). The LEARNING BRAIN dataset offers opportunities for multimodal analyses of the relationship between sensory experience, cognitive performance, and neural plasticity, as well as for methodological development in longitudinal and cross-modal neuroimaging research.

FINANCIAL SUPPORT: This project was funded by the National Science Centre grant 2018/30/E/HS6/00206.

## S2P62. VISUAL CORTEX RESPONSE TO DYNAMIC 3D CHECKERBOARD STIMULATION IN VIRTUAL REALITY: AN FNIRS STUDY IN YOUNGER AND OLDER ADULTS

Justyna Romanek, Mateusz Janecki, Jacek Jurkojć, Marta Chmura, Piotr Wodarski
*Department of Biomechatronics, Faculty of Biomedical Engineering, Silesian University of Technology, Gliwice, Poland*

INTRODUCTION: Checkerboard stimulation is a classical method for eliciting visual cortex activation. Three-dimensional (3D) environments have been shown to enhance cortical responses compared to two-dimensional stimuli. However, there is a lack of informations about age-related differences in occipital responses to dynamic 3D stimulation in virtual reality (VR).

AIM(S): The aim of this study was to evaluate visual cortex responses to a moving 3D checkerboard stimulus presented in VR and to compare response patterns in younger and older neurologically healthy individuals.

METHOD(S): The stimulus was an 8 Hz flickering checkerboard mapped onto a sphere in VR, moving alternately from the center to the left and right (10 repetitions per direction; 20 cycles total). Each 15 s stimulation block was followed by 20 s of a black screen. Visual cortex activity was recorded using fNIRS, and changes in oxygenated (HbO) and deoxygenated hemoglobin (HbR) were analyzed with a general linear model and random effects analysis.

RESULTS: In the younger group, dynamic 3D stimulation elicited robust bilateral occipital activation, with significant HbO increases and HbR decreases relative to baseline. Responses were spatially consistent at the group level. In older adults, activation was present individually but less spatially consistent and not stable at the group level. No global hemispheric dominance was observed. Greater variability in older adults aligns with reports of age-related functional reorganization and increased inter-individual variability.

CONCLUSIONS: Dynamic 3D checkerboard stimulation in VR effectively activates the visual cortex, particularly in younger adults. The greater spatial variability observed in the older group may reflect age-related functional reorganization. These findings provide a basis for further development of VR applications targeting visual stimulation, including future rehabilitation applications for patients with hemispatial neglect.

FINANCIAL SUPPORT: The publication was funded by Project No. FESL.10.25-IZ.01-07E7/23.

## S2P63. BRAIN MECHANISMS UNDERLYING IMPULSIVE RESPONSES DRIVEN BY TEMPORAL PREDICTABILITY: AN FMRI–EMG STUDY

Inga Korolczuk[1,2], Marion Royer D'Halluin[2,3,4], Bruno Nazarian[5], Boris Burle[2], Franck Vidal[2], Jennifer T Coull[2]

[1] *Department of Psychology, Medical University of Lublin, Lublin, Poland*

[2] *Centre for Research in Psychology and Neuroscience, Aix-Marseille University & CNRS, Marseille, France*

[3] *CHU Sainte-Justine Research Center, Montréal, Québec, Canada*

[4] *Department of Neurosciences, Université de Montréal, Montréal, Québec, Canada*

[5] *Aix-Marseille Université, Institut de Neurosciences de la Timone, Marseille, France*

INTRODUCTION: Previous work shows that temporal predictability speeds responding but increases impulsive errors during conflict tasks.

AIM(S): Here, we combined fMRI and EMG to identify the neural mechanisms underlying this effect.

METHOD(S): Twenty-four healthy adults performed a temporally cued Simon task. Visual pre-cues either predicted target onset (temporal cue) or provided no timing information (neutral cue). Participants responded to target shape (+/x) with left- or right-hand presses, while target location was either compatible or incompatible with the required response, inducing response conflict.

RESULTS: Behavioural results replicated earlier findings: temporal cues selectively increased fast impulsive errors on incompatible trials. fMRI analyses showed that temporal predictability recruited the left inferior parietal cortex (IPC) and left premotor cortex, regardless of response hand or compatibility. In contrast, response incompatibility activated the right putamen and right premotor cortex, independently of cueing. Critically, the interaction reflecting enhanced impulsivity for temporally predictable targets was associated with increased activity in left IPC.

CONCLUSIONS: This region, implicated in temporal attention and sensorimotor integration, may accelerate motor preparation based on temporal expectations, amplifying both correct and incorrect response tendencies. Importantly, overt errors capture only part of impulsive processing. EMG revealed that 16% of correct incompatible responses were preceded by subthreshold EMG activity in the incorrect hand ("partial errors"), which were rapidly suppressed. Temporal predictability significantly increased the frequency of these covert activations. Ongoing analyses aim to identify the neural substrates of partial errors to clarify how temporal predictability shapes the control of covert impulsive actions.

FINANCIAL SUPPORT: This work was supported by the National Science Centre of Poland grant (Sonatina 8, 2024/52/C/HS6/00009) awarded to Inga Korolczuk.

## S2P64. NEURAL BASIS UNDERLYING MENTAL ROTATION TASKS: A META-ANALYSIS OF FMRI STUDIES

Dana G.Stefanescu[1], Noelia Ventura-Campos[2], Zoe Falomir[3]

[1] *Neuropsychology and Functional Neuroimaging Research Group, Jaume I University, Spain*

[2] *Department of Education and Didactics of Specific Subjects, Jaume I University, Spain*

[3] *Computing Science Department, Umeå University, Sweden*

INTRODUCTION: Mental rotation (MR) is a cognitive process involving mental transformation of two- and three-dimensional objects, allowing accurate representation and recognition across orientations.

AIM(S): This meta-analysis aims to identify the neural bases of MR and to examine whether brain activation differs across stimulus types: three-dimensional figures (3D cubes), two-dimensional figures (alphanumeric characters and abstract shapes), and body-related stimuli.

METHOD(S): A PRISMA-guided systematic review identified 58 whole-brain fMRI studies (N=1131 participants). Analyses were conducted using Seed-based d Mapping with Permutation of Subject Images. Methodological details are available in OSF Registries (osf.io/zn2pd).

RESULTS: The main analysis revealed a robust bilateral activation pattern involving inferior and superior parietal gyri, precuneus, dorsolateral and superior frontal regions, precentral gyrus, and supplementary motor area. Additional engagement was observed in occipital and inferior temporal visual regions, as well as in cingulate and insular cortices. This configuration largely overlapped with the canonical dorsal attention network and supports the involvement of an extended attentional-visuomotor system underlying spatial transformation processes. In addition, stimulus type modulated the relative contribution of specific regions within this shared network. 3D stimuli preferentially recruited bilateral superior parietal and right frontal areas associated with spatial transformation and depth processing. In contrast, 2D stimuli showed a more

left-lateralized frontoparietal pattern, particularly involving inferior parietal and premotor regions. Hand stimuli additionally engaged the fusiform gyrus and dorsolateral frontal cortex, suggesting the contribution of body-related representations and motor simulation mechanisms.

CONCLUSIONS: Together, these findings indicate that MR relies on a common dorsal attentional network whose functional weighting varies according to stimulus characteristics.

FINANCIAL SUPPORT: Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital, Comunidad Valenciana (CIACIF/2023/284), Programa Fons Social Europeu Plus (FSE+).

## S2P65. STEEPER 1/F SPECTRAL SLOPE IS ASSOCIATED WITH STRONGER GAMMA PHASE-LOCKING DURING 40-HZ AUDITORY ENTRAINMENT: A CONDITION-SPECIFIC RELATIONSHIP ACROSS CORTICAL REGIONS

Daniel Borek[1], Gang Chen[2], Giovanni Pellegrino[3,4], Giorgio Arcara[3], Daniele Marinazzo[1]

[1] *Ghent University, Ghent*

[2] *National Institutes of Health, Bethesda*

[3] *IRCCS San Camillo Hospital, Venice*

[4] *Schulich School of Medicine, Western University, London, Ontario*

INTRODUCTION: Neural oscillations and the parametrized 1/f slope of the power spectrum are increasingly recognized as biomarkers of brain function, both potentially reflecting the local excitation-inhibition balance. While gamma-band synchrony is crucial for local circuit coordination, its relationship with the spectral slope remains poorly understood.

AIM(S): This study investigates whether the spectral exponent relates to stimulus-driven gamma synchronization during 40-Hz auditory steady-state responses (ASSR).

METHOD(S): We re-analyzed magnetoencephalography (MEG) data from 15 healthy participants during 40-Hz auditory stimulation, extracting spectral parameters (exponent and offset) and inter-trial phase coherence (ITPC) across 148 cortical regions. Using Bayesian multilevel regression with crossed subject and ROI random effects, we modeled both prestimulus and stimulus periods jointly to test the condition by exponent interaction on ITPC.

RESULTS: Auditory stimulation increased ITPC in auditory and temporal regions but did not alter the spectral exponent or offset. A positive relationship between spectral exponent and ITPC emerged during stimulation: steeper slopes were associated with stronger phase-locking to the 40-Hz stimulus, with this effect strongest in regions showing robust ASSR responses. Strong collinearity between exponent and offset limits our ability to disentangle exponent-specific effect.

CONCLUSIONS: These findings suggest that steeper slopes, potentially reflecting greater inhibitory influence, may provide the temporal scaffolding for precise neural synchronization with biological interpretation linked to excitation-inhibition balance, though methodological confounding in phase estimation cannot be ruled out. Integrated spectral and oscillatory measures may provide more sensitive markers of local circuit integrity than either measure alone.

## S2P66. RECORDING AND ANALYSIS OF VISUAL EVOKED POTENTIALS IN FREELY MOVING RATS

Čestmír Vejmola[1], Ivana Chrtková[2,4], Jan Hubený[2,5], Nina Miniariková[1], Martin Toman[1], Tomáš Páleníček[1,3]

[1] *Psychedelic Research Center, National Institute of Mental Health, Klecany, Czech Republic*

[2] *Applied Technology in Neuroscience, National Institute of Mental Health, Klecany, Czech Republic*

[3] *Third Faculty of Medicine, Charles University, Prague, Czech Republic*

[4] *Center for Advanced Studies of Brain and Consciousness, National Institute of Mental Health, Klecany, Czech Republic*

[5] *Department of Healthcare Technology, National Institute of Mental Health, Klecany, Czech Republic*

INTRODUCTION: Visual evoked potentials (VEPs) are widely used to assess visual pathway integrity in humans, yet their application in awake rodents remains limited due to methodological constraints. Most rodent VEP studies rely on anesthesia, which alters cortical dynamics and may confound translational interpretation.

AIM(S): We aimed to develop and validate a methodology for recording high-quality VEPs in awake rats and to characterize stimulus-specific response profiles

and adaptation dynamics across flash, pattern-reversal, and motion-onset paradigms.

METHOD(S): Ten male Long-Evans rats were implanted with 38 epidural electrodes covering the skull. Animals were head-fixed while standing on a freely rotating spherical treadmill, allowing natural locomotion. Visual stimuli (100× flash, 200× pattern-reversal, 300× motion-onset trials) were presented with jittered interstimulus intervals (~2.5 s). EEG was recorded at 1000 Hz and preprocessed in MNE-Python. VEP components were extracted from occipital electrodes (V7/V8). Adaptation of the P1 component was assessed using 20-trial sliding windows (95% overlap) and evaluated with Spearman correlation and permutation testing.

RESULTS: All stimulus types elicited robust, topographically specific responses maximal over the occipital cortex. Flash stimuli produced high-amplitude N40–P90 complexes, pattern-reversal evoked early P45–N60 peaks, and motion-onset generated N120–P250 responses. Adaptation analysis revealed a significant decrease in P1 amplitude and latency over time for flash stimuli ($p<0.001$), moderate modulation for motion-onset ($p<0.01$), and minimal change for pattern-reversal stimulation.

CONCLUSIONS: We demonstrate reliable VEP recording in awake rats without anesthesia, preserving physiological response properties. Distinct waveform morphology and adaptation patterns across stimulus types reflect functional differences in visual processing streams and support the translational utility of advanced visual paradigms in rodent models.

FINANCIAL SUPPORT: The work was supported by grants GAČR 23-07578K, SVV 260648/2024, ERDF CZ.02.01.01/00/22_008/0004643, LM2023049, the Cooperatio Neurosciences program, and the PSYRES foundation.

## S2P67. CEREBELLAR CONTRIBUTIONS TO AUTOMATIC AND CONTROLLED SEMANTIC MEMORY RETRIEVAL

Adam Kubinec, Rastislav Rovný, Igor Riečanský, Martin Marko

*Department of Behavioural Neuroscience, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovakia*

INTRODUCTION: Semantic memory retrieval is a fundamental cognitive function underlying adaptive behavior. Although accumulating evidence implicates the cerebellum in semantic processing and retrieval, its specific functional contribution remains unclear.

AIM(S): We tested the hypothesis that the right posterior cerebellum supports automatic (associative) memory retrieval rather than executive retrieval control.

METHOD(S): We conducted a double-blind, randomized controlled trial in which healthy adults completed free-associative (automatic) and dissociative (controlled) word retrieval tasks before and during either anodal or sham transcranial direct current stimulation (tDCS) targeting the right posterior cerebellum. The experiment additionally manipulated selection and switching demands, enabling a more process-sensitive assessment of retrieval dynamics.

RESULTS: Anodal cerebellar tDCS selectively impaired free-associative retrieval, while exerting no significant effect on retrieval control measures. Further analyses revealed that the disruption of free-associative performance was most pronounced in trials with cue words eliciting strong, dominant associations, compared to cues evoking a broader set of weaker word associations.

CONCLUSIONS: These findings provide evidence that the cerebellum contributes to automatic semantic retrieval, specifically by facilitating the activation of habitual, well-established conceptual associations. This role is consistent with the view of the cerebellum as a structure involved in the formation of habitual patterns and predictions across multiple domains, including memory, language, and higher cognition.

FINANCIAL SUPPORT: APVV-23-0145,
VEGA 2/0052/23, DoktoGrant APP0598.

## S2P68. CONTRIBUTION OF THE PREFRONTAL CORTEX TO CONTROLLED SEMANTIC RETRIEVAL

Adam Kubinec[1], Rastislav Rovný[1], Igor Riečanský[1,2], Martin Marko[1,3]

[1] *Department of Behavioural Neuroscience, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovak Republic*

[2] *Department of Psychiatry, Faculty of Medicine, Slovak Medical University in Bratislava, Bratislava, Slovak Republic*

[3] *Department of Applied Informatics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Bratislava, Slovak Republic*

INTRODUCTION: Semantic control refers to the cognitive and neural mechanisms that guide goal-directed retrieval of conceptual knowledge, often altered in neurodivergent populations. While neuroimaging consistently implicates the left lateral prefrontal cortex (PFC) in controlled semantic processing, its precise functional role remains unclear.

AIM(S): The study aimed to provide causal evidence for the role of the left lateral PFC in semantic control, particularly in executive inhibition of dominant conceptual associations during controlled retrieval.

METHOD(S): In a double-blind, randomized controlled trial, we applied anodal or sham transcranial direct current stimulation (tDCS) over the left lateral PFC to participants from two experimental groups (anodal: n=49; sham: n=50), who performed automatic-associative and controlled-dissociative word retrieval tasks before and during stimulation.

RESULTS: We found that anodal tDCS selectively impaired dissociative retrieval fluency—requiring executive inhibition of dominant associations—while leaving free-associative fluency unaffected. Crucially, prefrontal stimulation also increased the rate of conceptual intrusions during dissociative trials, and this effect substantially mediated the observed decline in retrieval fluency.

CONCLUSIONS: These findings provide causal evidence that the left lateral PFC supports proactive inhibitory control over semantic memory, suppressing context-inappropriate activations and intrusions to enable flexible, goal-relevant retrieval. Disruption of this mechanism impairs executive regulation of semantic retrieval, shedding new light on the neural architecture of semantic control and highlighting the potential of neuromodulatory approaches for addressing disordered conceptual processing.

FINANCIAL SUPPORT: APVV-23-0145, VEGA 2/0052/23, VEGA 2/0067/25, DoktoGrant APP0598.

## S2P69. PONG-CAPABLE NETWORK MOTIFS UNDER INCREASING COGNITIVE LOAD

Pablo Vidal Franco, Marcin Zagórski

*Institute of Theoretical Physics and Mark Kac Center for Complex Systems Research, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Cognitive tasks with varying levels of complexity can be solved efficiently by biological neural networks. However, we still lack understanding of how structural and functional features of these networks are affected by increasing the complexity of the goal function.

AIM(S): Here we address this open problem by investigating the emergence of minimal, overrepresented neural network motifs in a classic Atari game. Using a Genetic Algorithm to evolve ensembles of Artificial Neural Networks (ANN) to successfully play Pong we found the simplest network motif to consist of excitatory and inhibitory edge integrating the ball and paddle position into a single output node. Surprisingly, we found that this policy is successful for varying degrees of velocities and different paddle sizes.

METHOD(S): To answer whether more complex motifs occur as cognitive demands increase, we evolved additional ANNs under a version of Pong that introduces uncertainty by uniformly sampling angular velocity of the ball on every wall collision. Two ensembles were evolved, acting as Low- and High-Noise variants, and a dimensionality reduction of the structural features of successful ANNs was performed, followed by clustering and subgraph occurrence analysis. The successful ANNs were then re-evaluated under the same task using Wilson Lower Confidence Bound test to further filter optimal policies.

RESULTS: We made three observations: 1) the increased uncertainty produced consistently more complex motifs than earlier ensembles without uncertainty; 2) the minimal motif occurs as a subgraph in all other solutions; however, 3) in every ensemble one cluster consistently featured approximately 40% of non-standard solutions, that is, solutions that did not contain the simplest motif.

CONCLUSIONS: Hence, we speculate that increasing cognitive load results in the emergence of new network motifs that are qualitatively different from simple functional solutions.

FINANCIAL SUPPORT: This research was supported by the National Science Center, Poland (SONATA BIS, 2021/42/E/NZ2/00188).

## S2P70. ALTERED APPETITIVE CONDITIONING AND EXTINCTION IN COMPULSIVE SEXUAL BEHAVIOR: A NEUROIMAGING STUDY OF REWARD SYSTEM CONNECTIVITY

Jakub Wojciechowski[1,2], Małgorzata Draps[3], Xin Di[2], Bharat Biswal[2], Mateusz Gola[3,4]

[1] *Institute of Physiology and Pathology of Hearing, Warszawa, Poland*

[2] *Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, United States of America*

[3] *Institute of Psychology, Polish Academy of Sciences, Warszawa, Poland*

[4] *University of California San Diego, San Diego, United States of America*

INTRODUCTION: Compulsive Sexual Behavior Disorder (CSBD), included in the ICD-11, remains poorly understood in terms of its neural mechanisms. The Incentive Sensitization Theory of Addiction suggests that maladaptive attentional and motivational processes toward reward-related cues, coupled with impaired extinction, drive addictive behaviors through alterations in the brain's reward system.

AIM(S): This study aimed to investigate functional connectivity (FC) in the reward system during conditioning and extinction as well as intrinsic FC during resting state in individuals with CSB.

METHOD(S): Sixty-six right-handed heterosexual men (33 with CSB, 33 healthy controls) participated in fMRI scans while performing a conditioning and extinction tasks involving erotic, monetary, or neutral cues, as well as outside of task.

RESULTS: Results showed that both groups successfully acquired conditioned responses, but CSB participants exhibited stronger and more persistent cue preferences, particularly for erotic cues. Self-report ratings revealed heightened positive valence and arousal for erotic rewards in CSB participants, especially after conditioning. During extinction, CSB participants showed more robust extinction effects compared to controls for both erotic and monetary rewards. FC analyses revealed stronger ventral striatum–orbitofrontal cortex coupling for erotic cues during conditioning in CSB. During extinction, the coupling between ventral striatum and ventromedial prefrontal cortex was weaker in CSB, suggesting disrupted extinction. Interestingly, no differences were found in resting-state FC between groups.

CONCLUSIONS: These findings suggest that CSB involves enhanced conditioning, cue-reactivity, and impaired extinction learning, similar to other addictive behaviors. However, the lack of resting-state differences implies that the neurobiological mechanisms of CSB may differ from those seen in substance use disorders or involve more subtle, context-dependent neuroadaptations.

FINANCIAL SUPPORT: This study was supported by an Polish National Science Centre grant 2016/21/N/HS6/02635 and Fulbright Junior Research Award.

## S2P72. COMPARING AMYGDALA AND HIPPOCAMPAL CONTRIBUTIONS TO EPISODIC MEMORY RETRIEVAL FOLLOWING EMOTIONAL EXPOSURE IN YOUNGER AND OLDER ADULTS

Marianna Constantinou

INTRODUCTION: The amygdala and hippocampus are critical for processing episodic memories, particularly those with emotional significance. The amygdala binds emotional valence to memory representations while the hippocampus supports the retrieval of contextual information. In healthy ageing, the amygdala tends to maintain its structural integrity, whereas the hippocampus often shows functional decline, leading to difficulties in retrieving episodic details. Understanding how these regions interact during retrieval following emotional exposure is essential for identifying age-related neural adaptations.

METHOD(S): Using functional Magnetic Resonance Imaging (fMRI), neural activity was examined throughnrobust correlation analyses to track changes in synchronisation between the amygdala and hippocampus. Additionally, seed-based connectivity (SBC) analysis was used to map broader whole-brain interactions for each region.

RESULTS: In young adults, negative valence triggered an increase in neural synchronisation between the amygdala and hippocampus bilaterally. This was accompanied by increased hippocampal functional connectivity (FC) with parietal and frontal regions, reflecting an adaptive response to the cognitive load of negative valence exposure. Conversely, older adults showed a significant decrease in synchronisation over time, particularly in the right hemisphere, consistent with a "crunch" effect indicating insufficient compensatory capacity. Their FC was restricted to posterior occipital regions, indicating reliance on sensory-visual processing.

CONCLUSIONS: The findings reveal that while both the amygdala and hippocampus are essential for memory retrieval, they facilitate memory through distinct neural strategies across the lifespan. Younger adults utilise broad, hippocampal-led networks to overcome negative emotional interference, whereas older adults rely on localised posterior pathways that may contribute to age-related declines in memory accuracy.

## S3P01. FROM RIGID TO FLEXIBLE: REDOX-ACTIVE MACROMOLECULAR SYSTEMS FOR EMULATING NEURAL PLASTICITY IN ORGANIC ELECTRONICS

Anna Kostecka, Wojciech Wieczorek, Krystian Sokołowski, Tomasz Mazur, Konrad Szaciłowski, Michał Szuwarzyński

*Academic Center for Materials and Nanotechnology, AGH University of Kraków, Kraków, Poland*

INTRODUCTION: Bio-inspired computing requires hardware mimicking the processing power and physical nature of the biological brain. Unlike rigid silicon electronics, organic memristors offer a unique combination of analog switching (plasticity) and mechanical flexibility, making them ideal "artificial synapses" for bio-integrated electronics. However, the computational fidelity of such devices depends critically on the nanoscale engineering of the polymer-metal interface.

AIM(S): This study evaluates poly(glycidyl methacrylate) (PGMA) brushes as a platform for emulating neural functions. We investigate how the top electrode deposition technique (Thermal Evaporation *vs.* Magnetron Sputtering) and synthesis method influence resistive switching and the ability to mimic synaptic plasticity in both rigid and flexible systems.

METHOD(S): PGMA brushes were synthesized via surface-initiated polymerization on rigid (ITO/glass) and photopolymerization on flexible (PET/ITO) substrates to suit soft electronics. Functionalization with redox-active aminoferrocene introduced charge-trapping states, alongside control amines (hexylamine, fluorobenzylamine). Top gold electrodes were deposited via low-energy Thermal Evaporation or high-energy Magnetron Sputtering. Morphology and composition were characterized by AFM, XPS, and IR spectroscopy, followed by electrical measurements to assess neuromorphic behavior.

RESULTS: Analysis reveals that the interface formation technique governs the symmetry and linearity of switching dynamics (learning rules). We compare deposition routes to determine optimal stability conditions. Furthermore, the performance of photopolymerized flexible systems is assessed under mechanical bending to verify their potential for wearable neuromorphic applications.

CONCLUSIONS: This work establishes design rules for organic artificial synapses. By analyzing fabrication parameters, we provide essential insight into developing thin-film macromolecular systems tailored for efficient and flexible neural function emulation.

FINANCIAL SUPPORT: This study was funded by National Science Centre (Poland) [Sonata Bis Grant no. 2021/42/E/ST4/00290].

## S3P02. *IN VIVO* BRAIN MRS THERMOMETRY: STABILITY OF MEASUREMENT ANALYSIS

Marcin Sińczuk[1], Jacek Rogala[2], Piotr Bogorodzki[1]

[1] *Mossakowski Medical Research Institute, Polish Academy of Sciences, Warszawa, Poland*

[2] *Centre for Research on Culture, Language, and Mind, University of Warsaw, Warszawa, Poland*

INTRODUCTION: Magnetic resonance spectroscopy (MRS) thermometry is an innovative method for non-invasive, localized brain temperature measurement, but its stability across repeated visits remains insufficiently researched.

AIM(S): In this study, we aimed to assess the stability of MRS thermometry by testing whether temperature changes after prolonged fMRI are reproducible across two visits.

METHOD(S): In vivo data from forty-three healthy adults (22F, 21M) were acquired across two visits (median interval: 200 days). At each visit, two 1HMRS PRESS spectra were acquired from each subject: one before and after 30-minute fMRI session. Temperature was estimated using previously acquired calibration data sets and water-metabolite chemical shift differences between suppresed (WS) and unsupressed (WU) water and N-acetylaspartate (NAA), creatine (Cr), and choline (Cho) used as references.

RESULTS: Across all methods and both visits, temperature change was consistently negative, indicating post-fMRI cooling. Measured mean cooling was approximately -0.25°C for WS (NAA/Cr/Cho; all $p<0.001$) and -0.16°C for WU (NAA/Cr/Cho; all $p<0.001$). Cooling occurred in ~80% of WS and ~65% of WU observations. Absolute temperature measurements showed systematic offset with WU yielding higher values by ~0.6°C. Sensitivity analyses confirmed the direction and significance of cooling.

CONCLUSIONS: Prolonged fMRI is associated with small but robust decrease in brain temperature across all used methods. WS provides higher stability and larger apparent cooling, while absolute temperature measurements depend on acquisition type. We believe that using MRS thermetry for longitudinal studies is feasible.

FINANCIAL SUPPORT: This study was part of the grant NCBR nr POIR.01.01.01-00-0178/15-00.

## S3P03. AMYGDALA, BUT WHICH ONE? A COMPARATIVE ANALYSIS OF DIFFERENT AMYGDALA MASKS

Sylwia Adamus[1], Małgorzata Draps[2], Piotr Suffczyński[3]

[1] *Infant Jesus Teaching Hospital, Warszawa, Poland*

[2] *Lab of Clinical Neuroscience, Institute of Psychology, Polish Academy of Sciences, Warszawa, Poland*

[3] *Faculty of Physics, Biomedical Physics Division, University of Warsaw, Warszawa, Poland*

INTRODUCTION: The amygdala is a paired, subcortical structure which plays a role in multiple processes related to emotional processing. Its neuroimaging in vivo is a major challenge due to its small size and complex internal structure. As a result, there is a vast variety of masks which can be used to define its boundaries. This has been a major drawback in studies regarding amygdala's volume alterations.

AIM(S): The aim of this study was to compare different amygdala masks on the level of the unparcellated structure, as well as its subparts.

METHOD(S): Resting-state functional magnetic resonance imaging data from 155 healthy individuals with no underlying neurological or psychiatric conditions was acquired from the OpenNeuro repository. The data was preprocessed and denoised using CONN Functional Connectivity Toolbox and a group-level resting-state functional connectivity (rs-FC) analysis was conducted for three different amygdala masks. Next, a parcellation pipeline was applied to the blood oxygen level dependent signal of the voxels within the amygdala as defined by each of the masks.

RESULTS: The rs-FC analysis showed that despite multiple connections present regardless of the used mask, connections with several brain regions were unique to particular masks. This was most prominent for the left amygdala when defined with the mask by Dominik Bach et al (2011), compared to a popular mask of similar size from the Harvard-Oxford atlas. The amygdalae were divided into two subdivisions – the dorsomedial (DM) and the ventrolateral (VL). For the Bach mask the DM/VL size ratio was remarkably smaller for both amygdalae compared to other masks.

CONCLUSIONS: To conclude, even small changes in the size and spatial position of the chosen amygdala mask can result in markedly different patterns of rs-FC and internal structure. As the amygdala is known to be involved in the development of multiple mental health conditions, this might provide an explanation why many studies in clinical groups have failed to obtain consistent results.

FINANCIAL SUPPORT: Not applicable.

## S3P04. MODELLING THE ANATOMY OF ROSTROMEDIAL TEGMENTAL NUCLEUS (RMTG)

Kaja Wróblewska[1], Gniewosz Drwięga[1,2], Martyna Gorkowska-Nosal[2,3], Kamil Pradel[4], Tomasz Błasiak[2]

[1] *Department of Bioinformatics, Jagiellonian University, Kraków, Poland*

[2] *Department of Neurophysiology and Chronobiology, Jagiellonian University, Kraków, Poland*

[3] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

[4] *Institute for Systems Physiology, University of Cologne, Cologne, Germany*

INTRODUCTION: Rostromedial Tegmental Nucleus (RMTg), or the tail of Ventral Tegmental Area (VTA), is a midbrain structure considered one of the key regulators of the reward system, responsible for modulating the level of motivation and reward-driven behaviour. Despite its importance, the anatomical boundary of RMTg remains poorly defined and is not included in commonly used rat brain atlases.

AIM(S): This study aimed to determine the structure of the RMTg and define its boundaries on the Paxinos and Watson stereotaxic maps.

METHOD(S): The identification of the structure was carried out using immunohistochemical staining in 7 rats. The regulatory protein FOXP1 was utilised, as its expression was shown to be specific to the RMTg neurons in the midbrain.

RESULTS: Based on FOXP1-positive neuron distribution, a three-dimensional model was generated. Precise coordinates for the RMTg were extracted across multiple coronal planes. These spatial data allowed for the mapping of averaged RMTg boundaries onto corresponding atlas templates, resulting in a representative anatomical model.

CONCLUSIONS: These findings allow for more precise localisation of the RMTg, which can improve the methodological quality of future studies on the role of this structure in the regulation of the reward system.

FINANCIAL SUPPORT: Financial support: Preludium 2022/45/N/NZ/03171.

## S3P05. HISTOLOGY-INFORMED TRACTOGRAPHY BENCHMARK AND CONSTRAINT LEARNING USING CONDITIONAL VARIATIONAL AUTOENCODER AND REGISTERED PROTEOLIPID PROTEIN (PLP) MYELIN HISTOLOGY

Monika Pytlarz, Jan K. Argasiński

*Sano Centre for Computational Medicine, Kraków, Poland*

INTRODUCTION: Diffusion MRI tractography is highly sensitive to modeling choices and tracking heuristics, yet objective validation is limited by the scarcity of microscopic ground truth aligned to MRI. We present a histology-informed tractography benchmark in post-mortem human corpus callosum (CC) white matter using diffusion MRI (dMRI) and co-registered proteolipid protein (PLP) myelin histology from the Oxford Digital Brain Bank.

AIM(S): We aim to introduce a histology-informed benchmark for tractography in post-mortem white matter in CC and test whether PLP-derived maps can be predicted from dMRI.

METHOD(S): We analysed three CC specimens and regions of interest (ROIs) in lateral/midline CC, corticospinal tract, and centrum semiovale. Using released MRI-histology registrations and PLP maps, we compared spherical deconvolution, tensor-based, FACT, and null-distribution tractography. Streamlines were mapped to histology and evaluated by tissue coverage and axial angular error *vs.* local PLP orientations. We also predicted PLP orientation-dispersion from registered MRI orientation-dispersion and mean diffusivity using leave-one-sample-out regression and a small U-Net. Exploratively, we trained a conditional variational autoencoder (cVAE) to predict tissue-informed orientation distributions from MRI and derive tract-level anatomical constraint scores.

RESULTS: Tractography algorithm performance varied across specimens and methods. Null-distribution tractography gave control angular errors of ~45°. Selected methods showed lower angular error with moderate coverage. In the CC ROI, the U-Net showed moderate correspondence with PLP targets, whereas label-shuffled controls were near zero or negative. Exploratory cVAE analyses supported feasible PLP-informed orientation-distribution prediction and tract-level scoring on held-out data.

CONCLUSIONS: This work provides a reproducible microscopy-referenced benchmark for tractography in human CC white matter and a proof-of-principle MRI-to-histology prediction framework.

FINANCIAL SUPPORT: Horizon 2020 (Grant No. 857533), FNP (MAB PLUS/2019/13), and the Polish Ministry of Science and Higher Education (MEiN/2023/DIR/3796).

## S3P06. EXPANDING THE EBRAINS DIGITAL ATLASING ECOSYSTEM: INTEGRATING THE COMMON MARMOSET BRAIN TEMPLATE

Piotr Majka[1], Shi Bai[2], Adam Datta[1], Karolina Łabuszewska[1], Marcin Syc[1], Tomasz Walkiewicz[1], Marcello Rosa[2]

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

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

INTRODUCTION: EBRAINS is a leading platform for advancing neuroscience research, providing extensive support for rodent and human-oriented datasets. However, it currently supports only one non-human primate (NHP) species, despite the critical role NHPs play in bridging the transnational gap between rodent models and human studies. From a technical standpoint, the platform lacks a standardized procedure for incorporating atlases of additional animal species.

AIM(S): To address this limitation, we propose extending the EBRAINS platform by integrating an atlas of the common marmoset brain, termed Marmoset@EBRAINS. The atlas is accompanied with additional datasets, including neuronal distribution and cellular-level connectivity. Additionally, we demonstrate how EBRAINS users can map their own datasets onto the atlas using the platform's digital atlasing tools.

METHOD(S): The proposed atlas is derived from the Nencki-Monash marmoset brain template, a morphological average of 20 marmoset brains. The template combines high-resolution cytoarchitectural information with the isotropic resolution of MR-based templates. The atlas is implemented via Siibra-Explorer, the native EBRAINS atlas browser, while accompanying datasets were curated using the OpenScienceData workflow.

RESULTS: We implemented the Marmoset@EBRAINS atlas as a hosted resource on the platform. To facilitate this integration, we also extended the Siibra-Explorer plugin system to support features specific to our atlas. Further, we provided four curated, FAIR-compliant datasets that are fully integrated into the EBRAINS Knowledge Graph and registered with the openMINDS metadata framework.

CONCLUSIONS: This project lays the foundation for integrating the marmoset as a model species within EBRAINS. By providing a framework that enables other research groups to contribute data, it substantially broadens the platform's potential user base. Finally, this work marks an important step toward generalizing EBRAINS' atlasing tools and increasing the platform's versatility.

FINANCIAL SUPPORT: EBRAINS 2.0 (101147319, Financial Support to Third Parties, FSTP).

## S3P07. DO DYADS SYNCHRONIZE OR ARE WE JUST IMAGINING IT? NOVEL PIPELINE FOR DYADIC MOVEMENT ANALYSIS

Aleksandra Kołakowska[1], Julia Słowicka[1], Grzegorz Kaliński[1], Aleksandra Hamny[1], Jarosław Żygierewicz[2], Agnieszka Pluta[1,3]

[1] *Faculty of Psychology, University of Warsaw, Warszawa, Poland*

[2] *Faculty of Physics, University of Warsaw, Warszawa, Poland*

[3] *Bioimaging Research Center, Institute of Physiology and Pathology of Hearing, World Hearing Center, Warszawa, Poland*

INTRODUCTION: Biobehavioral synchrony in dyadic interaction refers to the coordinated, mutual, and reciprocal alignment of physiological states, behaviors, or emotional expressions. The study followed Self- and Co-Regulation (SECORE) protocol that captures the phases of the interaction at different levels of regulation and arousal.

AIM(S): To build a research pipeline that detects dyadic movement from video recordings of the experiment.

METHOD(S): The training dataset for the predictive model consists of the object detection data from the Common Object in Context (COCO) dataset and corresponding age labels. For each person in a video frame, the algorithm infers the position of 17 body keypoints and age group classification. The computer vision pipeline outputs additional measures, i.e., distance, velocity, keypoint speed, movement entropy, and similarity in movement direction (cosine similarity).

RESULTS: The algorithm achieved a high average classification accuracy when using five-fold stratified cross-validation on the test COCO dataset. We compared 30 caregiver and typically developing child dyads (TD-TD) with 20 caregiver child dyads in which the child had autism spectrum disorder (TD-ASD). Preliminary results suggest that children in the ASD group maintained distance from their caregiver more often than typically developing children. Interestingly, the mean distance in the TD-TD dyads was significantly greater than the distance in the TD-ASD dyads. What is more, TD-TD dyads had a higher frequency of interaction, with both the child and the caregiver approaching each other. Children in both groups moved more towards the caregiver with greater magnitude variation in TD-TD dyads.

CONCLUSIONS: Taken together, these preliminary findings point to group-specific differences in proximity management and interaction dynamics that warrant further investigation in larger samples. Our results showed that the measures obtained from the pipeline can be used to analyse the dyadic synchrony which has a real impact on the field.

FINANCIAL SUPPORT: The research was supported by the European Union under the Horizon-Widera Europe program (grant agreement No. 101159414).

## S3P08. DECOMPOSITION OF PHENOTYPIC HETEROGENEITY IN AUTISM REVEALS UNDERLYING CHANGES IN OCULOMOTOR PATTERNS

Mikołaj Iwo Turczyniak[1], Cemal Koba[2], Jan K. Argasiński[2], Marcin Leszczyński[3]

[1] *Doctoral School of Social Sciences, Myślenice, Polska*

[2] *SANO – Centre for Computational Personalised Medicine, Kraków, Poland*

[3] *Department of Cognitive Science, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Eye-tracking measures provide an objective window into visual attention and have been increasingly proposed as potential biomarkers of autism spectrum disorder (ASD) diagnosis. However, heterogeneity within ASD and methodological inconsistencies across studies limit the interpretability and reproducibility.

AIM(S): In this study, we investigated eye-movement characteristics across clinically defined ASD subgroups using a large, multi-class dataset to predict the phenotypic heterogeneity in ASD classification.

METHOD(S): Eye-tracking data were analyzed from over 3,300 participants (including 1,042 ASD patients) exposed to three standardized movie stimuli

(free-viewing) using fixation, saccade, and blink metrics. Four ASD subgroups were defined based on clinical profiles: Moderate Challenges, Broadly Affected, Social/Communication, and Developmental Disorders. Children without positive scores in CBCL, RBS, and SQL tests were used as a control group.

RESULTS: To control for age and gender confounds, ASD subgroups were matched 1-to-1 (914-to-914 subjects) with neurotypical controls using nearest-neighbor matching within a ±1-year age tolerance and exact gender correspondence, leaving 1472 control participants. Eye-tracking metrics were compared across groups using multi-model ANOVA with Tukey HSD post-hoc analysis. ASD subgroups exhibited significant differences in fixation duration relative to matched controls ($p<0.05$), suggesting distinct oculomotor profiles across ASD subtypes. Precision of 0.97, suggesting that oculomotor measures might be diagnostically useful. Quantifying the minimal amount of eye tracking needed to classify phenotypes using free-viewing tasks, we found that as little as 10 minutes is required to achieve 0.97 precision and 0.98 recall.

CONCLUSIONS: We demonstrate that phenotypes show selective and specific alternation of oculomotor patterns. These results highlight the use of eye tracking in ASD diagnostics.

FINANCIAL SUPPORT: This project was funded by National Science Center, Poland, grant no 2024/55/D/NZ5/02998. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 857533 and from the International Research Agendas Programme of the Foundation for Polish Science No MAB PLUS/2019/13. The project was created within the project of the Minister of Science and Higher Education "Support for the activity of Centers of Excellence established in Poland under Horizon 2020" on the basis of the contract number MEiN/2023/DIR/3796. We acknowledge Polish high-performance computing infrastructure PLGrid (HPC Center: ACK Cyfronet AGH) for providing computer facilities and supportwithin computational grant no. PLG/2025/01828.

## S3P09. GLOBAL AND REGIONAL DIFFERENCES IN THE APERIODIC EXPONENT OF THE EEG POWER SPECTRUM BETWEEN CHILDREN AND ADOLESCENTS WITH AND WITHOUT ADHD

Sheyla Andalia-Crespo, Brenda Y Angulo-Ruíz, Vanesa Muñoz, Elena I. Rodríguez-Martínez, Carlos M. Gómez

INTRODUCTION: The aperiodic exponent of the EEG power spectrum has been proposed to reflect aspects of cortical excitation–inhibition balance and neural maturation. Pediatric ADHD studies show inconsistent findings, potentially due to age and scalp distribution differences. Examining global and regional patterns while controlling for developmental factors may clarify these inconsistencies.

AIM(S): To investigate global and regional differences in the aperiodic exponent between children and adolescents with ADHD and neurotypical controls, accounting for age and sex.

METHOD(S): Resting-state EEG was recorded for 3 minutes with eyes open in 93 participants aged 6–17 years (52 controls, 41 ADHD). The aperiodic exponent was calculated using Fitting Oscillations and One-Over-f (FOOOF) (1–45 Hz). A univariate ANCOVA tested group differences using the mean exponent across 30 electrodes, controlling for age and sex. A linear mixed-effects model examined regional exponents, with fixed effects for group, anterior–posterior axis, lateralization, age, and sex, and subject as a random effect. Analyses were performed in Matlab R2021b, SPSS 25, and JASP 0.95.4.

RESULTS: The ANCOVA revealed a significant group effect, with ADHD showing a lower exponent than controls after controlling for age and sex, indicating a flatter power spectrum. In the mixed-effects model, significant effects were observed for group, lateralization, anterior–posterior axis, and age; sex was not significant. These results indicate that group differences are present globally and modulated by scalp topography.

CONCLUSIONS: Children and adolescents with ADHD exhibit a reduced aperiodic exponent compared to controls, independent of sex and age. Significant spatial effects highlight the importance of considering scalp distribution. These findings support the aperiodic exponent as a marker of altered neural information processing and excitation–inhibition balance in ADHD.

FINANCIAL SUPPORT: This research was funded by Agencia Estatal de Investigación (AEI), grant number PID2022–139151OB-I00 (FEDER funds).

## S3P10. ELECTROPHYSIOLOGICAL AND ANATOMICAL ALTERATIONS OF VENTRAL DENTATE GYRUS CIRCUITRY IN A RAT MODEL OF AUTISM SPECTRUM DISORDER

Aleksandra Nogaj[1,2], Kacper Kula[1], Aleksandra Trenk[1], Anna Guguła[1], Kinga Przybylska[1,2], Anna Błasiak[1]

[1] *Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

[2] *Doctoral School of Exact and Natural Sciences, Faculty of Biology, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Autism spectrum disorder (ASD) is characterized by social impairments, anxiety, and disturbances in hippocampal processing. The ventral dentate gyrus (vDG), a region involved in social memory processing and pattern separation, is particularly susceptible to circuit-level imbalance. Alterations in inhibitory interneurons, especially parvalbumin (PV+) and somatostatin (SST+) cells, as well as neuromodulatory inputs to DG may contribute to abnormalities observed in ASD.

AIM(S): The present study examined functional and anatomical changes in vDG neurons in a rat model of ASD, focusing on granule cells, interneuron subtypes, and cells expressing the relaxin-3 (RLN3) receptor-RXFP3.

METHOD(S): ASD-like phenotypes were induced by prenatal valproic acid (VPA) exposure (500 mg/kg, E12.5) in Sprague-Dawley rats. Intrinsic membrane properties and synaptic activity of vDG granule cells were evaluated using whole-cell patch-clamp recordings. Molecular profile of vDG cells was assessed using multiplex RNAscope targeting PV, SST, vGAT1, vGLUT2, and RXFP3 mRNA. Immunohistochemistry was used to assess PV+ and SST+ cell density in vDG and RLN3+ neurons in the nucleus incertus.

RESULTS: Granule cells in VPA-exposed animals displayed enhanced neuronal gain, indicating increased excitability, while passive membrane parameters remained unaffected. Female VPA rats showed a reduced density of SST+ interneurons, whereas PV+ interneuron numbers were not affected. RXFP3 expression was largely confined to vGAT1-positive neurons, with frequent co-expression in SST+ cells.

CONCLUSIONS: Our findings demonstrate heightened excitability of vDG granule cells accompanied by selective vulnerability of SST interneurons in VPA females. RXFP3 expression in SST+ and other inhibitory vDG neurons suggests that RLN3 signaling may influence inhibitory networks and contribute to hippocampal dysfunction in ASD.

FINANCIAL SUPPORT: National Science Centre, Poland (UMO-2023/49/B/NZ4/01885; UMO2024/53/N/NZ4/03919).

## S3P11. 5-HT$_2$A AND SIGMA-1-MEDIATED MODULATION OF NEUROINFLAMMATION AND BLOOD–BRAIN BARRIER INTEGRITY

Ying Yu[1], Wiesław J. Cubała[2], Aleksandra Rutkowska[1]

[1] *Division of Anatomy and Neurobiology, Medical University of Gdańsk, Gdańsk, Poland*

[2] *Department of Psychiatry, Medical University of Gdańsk and University Clinical Centre, Gdańsk, Poland*

INTRODUCTION: Psychedelics such as N,N Dimethyltryptamine (DMT) are widely studied for their psychoactive properties, yet their direct effects on neuroinflammation and blood–brain barrier (BBB) function remain incompletely understood.

AIM(S): The aim of this study was to determine whether DMT modulates inflammatory signalling and endothelial pproperties relevant to BBB function and to identify the receptor mechanisms underlying these effects.

METHOD(S): Organotypic cerebellar slice cultures were exposed to lysophosphatidylcholine-induced demyelination and treated with DMT, a dual 5-HT$_2$A or Sigma-1 receptor agonist, in the presence or absence of receptor specific antagonists. NF-κB signalling and cytokine release were analysed and BBB-related gene expression was quantified. In parallel, a human tri-cell BBB model was exposed to inflammatory cytokines in the presence of DMT and antagonists.

RESULTS: DMT attenuated NF-κB signalling and reduced pro-inflammatory cytokine release in the cerebellar slices. In the same model, DMT reversed LPC-induced VCAM1 induction, did not rescue Occludin expression but further enhanced VE-cadherin indicating anti-inflammatory effects on the endothelium. In the human BBB model, DMT also exerted anti-inflammatory effects while differentially modulating junctional proteins. The observed effects were largely abolished by 5-HT$_2$A or Sigma-1 receptor antagonists, supporting receptor-specific mechanisms.

CONCLUSIONS: Together, these findings demonstrate that DMT exerts anti-inflammatory and immunomodulatory effects in the vascular compartment, contributing to the preservation of BBB integrity via 5-HT$_2$A and Sigma-1 signalling. Our results provide mechanistic insight into the cellular actions of psychedelics and suggest potential relevance for neuroimmune and neurodegenerative disease contexts.

FINANCIAL SUPPORT: no.

## S3P12. BEYOND NEURONS: IMMUNOREGULATORY ACTIONS OF PSYCHEDELICS IN NEUROINFLAMMATION AND DEMYELINATION

Ying Yu[1], Wiesław J. Cubała[2], Aleksandra Rutkowska[1]

[1] *Division of Anatomy and Neurobiology, Medical University of Gdańsk, Gdańsk, Poland*

[2] *Department of Psychiatry, Medical University of Gdańsk and University Clinical Centre, Gdańsk, Poland*

INTRODUCTION: Psychedelics are emerging as modulators of neuroinflammation, yet their mechanisms remain poorly understood.

AIM(S): In our lab, we investigate five compounds—DMT, 5-MeO-DMT, LSD, ketamine and ibogaine—chosen for their activity at 5-HT2A and/or Sigma-1 receptors, key players in neuroimmune signalling. These molecules exhibit diverse receptor profiles, acting as agonists, partial agonists or antagonists across serotonergic, NMDA, adrenergic, opioid and sigma receptors, influencing processes such as cognition, neurogenesis and immune function.

METHOD(S): Here, we tested anti-inflammatory properties of selected psychedelics in the organotypic cerebellar slice model, primary oligodendrocyte progenitor cells (OPCs) and human blood-brain barrier model.

RESULTS: DMT reduced LPC-induced NF-κB signalling and cytokine release, protected against demyelination and inhibited inflammation-driven OPC activation in organotypic cerebellar slices. On endothelial cells, DMT counteracts VCAM1 induction and selectively modulates junctional gene expression.

CONCLUSIONS: DMT acts primarily as an immune regulator. These insights provide a foundation for understanding how psychedelics may shape neuroinflammatory pathways and guide future therapeutic strategies.

## S3P13. KETAMINE PREVENTS TIME-DEPENDENT CHANGES IN SERUM BDNF CONCENTRATIONS AND ANHEDONIA INDUCED BY STRESS IN WISTAR–KYOTO RATS

Natalia Gaik, Emil Dzierkacz, Paulina Pabian, Karolina Dobranowska, Piotr Gruca, Mariusz Papp, Agata Faron-Górecka

*Department of Pharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Chronic mild stress (CMS) is commonly used to model anhedonia in rodents, but stress-sensitive strains may exhibit distinct temporal neurobiological responses. Wistar–Kyoto (WKY) rats are characterized by high stress reactivity and stable affective traits, making them suitable for studying the interaction of stress and antidepressant treatment over time.

AIM(S): Aim of the study was to determine whether repeated ketamine administration modulates time-dependent changes in serum brain-derived neurotrophic factor (BDNF) levels induced by CMS in stress-sensitive WKY rats and whether these changes relate to anhedonic behavior.

METHOD(S): Two independent cohorts of male WKY rats were subjected to the CMS protocol. During CMS, animals received weekly injections of ketamine or saline for 9–10 weeks. Anhedonia was assessed using a sucrose intake test. Serum BDNF levels were measured 24 or 48 hours after the last injection using ELISA and expressed relative to control values.

RESULTS: CMS consistently reduced sucrose consumption, confirming an anhedonic phenotype. Stress-related alterations in serum BDNF were observed only in saline-treated animals. After 24 hours, CMS decreased BDNF levels in stressed saline-treated rats, whereas no difference was observed in ketamine-treated group. After 48 hours however, CMS induced a significant increase in serum BDNF concentration in stressed and saline-treated rats. In contrast, BDNF levels in ketamine-treated animals did not differ from control values at both time points. A significant correlation between sucrose consumption and serum BDNF levels was observed only after 24 hours.

CONCLUSIONS: The results show that chronic stress causes changes in serum BDNF levels in WKY rats, and the nature of these changes depends on the time of measurement. Repeated ketamine administration prevents stress-induced changes in BDNF concentrations. Our results emphasize that the timing of measurement is crucial when interpreting peripheral neurotrophic markers in stress-sensitive animal models.

FINANCIAL SUPPORT: These studies were financed by statutory activities of the Department of Pharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences in Kraków.

## S3P14. CHRONIC STRESS AND KETAMINE AFFECT DOPAMINE D2 RECEPTORS IN A REGION-SPECIFIC MANNER IN WISTAR–KYOTO RATS

Emil Dzierkacz, Gaik N, Pabian P, Dobranowska K, Gruca P, Papp M, Faron-Górecka A

*Department of Pharmacology, Maj Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: The dopaminergic system plays an important role in regulating motivation and stress responses, which are impaired in depression. Wistar–Kyoto (WKY) rats are particularly sensitive to stress and are therefore often used as a model for affective disorders.

METHOD(S): In this study, male WKY rats were subjected to chronic mild stress (CMS). During CMS, the animals received weekly ketamine (10 mg/kg, i.p.) or saline. Brains were collected 24 hours after the end of CMS and the last ketamine administration. The availability of dopamine D2 receptors was assessed by autoradiography using [3H]spiperone in selected brain regions, including the prefrontal cortex, striatum, nucleus accumbens (core and shell), hippocampus, substantia nigra, and amygdala.

RESULTS: Significant changes in D2 receptor binding were observed, which depended on both stress exposure and ketamine administration, as well as the brain region analysed. In some structures, chronic stress was associated with changes in D2 receptor availability, while in others, ketamine modified binding levels independently of stress. The greatest differences were observed in structures associated with motivation and reward regulation, particularly in the nucleus accumbens, especially its shell part. The obtained results indicate that the observed changes in D2 receptors are not only due to stress, but are the effect of the interaction between chronic stress and repeated exposure to ketamine.

CONCLUSIONS: This study demonstrates that in WKY rats, chronic stress and ketamine affect the dopaminergic system in a complex manner that depends on brain structure.

FINANCIAL SUPPORT: These studies were financed from the statutory activities of the Department of Pharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences in Kraków.

## S3P15. INVESTIGATION OF THE ANXIOLYTIC AND NEUROCHEMICAL MECHANISM OF PSILOCYBIN MICRODOSING IN RATS

Maja Gramatyka, Agnieszka Bysiek, Krystyna Gołembiowska

*Department of Pharmacology and Brain Biostructure, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Psilocybin, a member of the serotonergic psychedelic class, is currently attracting interest due to its suggested therapeutic effects on anxiety and mood disorders. An alternative to clinically effective single doses of psychedelics is the use of small doses (a one-tenth of the therapeutic one) administered over a longer period of time, so called "microdoses". Microdosing can provide therapeutic benefits, but with a low risk of adverse effects, such as hallucinations or perceptual distortions.

AIM(S): This study aimed to investigate the effectiveness of psilocybin microdosing on rat behaviour and oxidative DNA damage in the rat brain.

METHOD(S): Psilocybin at a dose of 0.06 mg/kg (sc) was administered to adult Wistar-Han rats for 2 weeks at 2-day intervals. Two days after drug termination several behavioral tests such as the Wet Dog Shakes test (WDS), the Elevated Plus Maze test (EPM) and the Light/Dark Box test (LDB), the Open Field Test (OF) and the Novel Object Recognition test (NOR) were performed. In addition, comet assay in the nuclear fraction obtained from the frontal cortex and hippocampus of rats was carried out.

RESULTS: Psilocybin induced a mild but significant hallucinogenic effect in the WDS test, demonstrated an anxiolytic effect in the EPM, but not in the LDB test. A small but statistically significant increase in motor activity and compulsive behavior in the OF test was observed. Furthermore, increased exploration of the illuminated central zone of the OF and no effect in the cognitive functions in the NOR test was obtained. Any oxidative damage of DNA was noted in the nuclear fractions of the frontal cortex and hippocampus of rats.

CONCLUSIONS: Results of our study indicate that psilocybin administered in a microdosing regimen may have a beneficial effect on the level of anxiety and exploratory activity of animals, does not cause the damage of genetic material, but induces psychotomimetic effect.

FINANCIAL SUPPORT: This research was funded by statutory funds of the Maj Institute of Pharmacology, Polish Academy of Sciences.

## S3P16. THE EFFECT OF PSILOCIN ON NEUROTRANSMITTERS RELEASE IN THE CLAUSTRUM AND ON RAT BEHAVIOR

Zuzanna Kościuk[1,2], Izabela Szpręgiel[1], Agnieszka Bysiek[1], Krystyna Gołembiowska[1]

[1] *Department of Pharmacology and Brain Biostructure, Unit II, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Institute of Zoology and Biomedical Research, Department of Biology, Jagiellonian University, Kraków, Poland*

INTRODUCTION: The claustrum is a subcortical structure densely expressing 5-hydroxytryptamine 2A receptors (5-HT2AR). Despite its hypothesized involvement in the effects of serotonergic psychedelics, the neurochemical impact of these substances on claustral neurotransmission remains unexplored.

AIM(S): This study aimed to investigate how psilocin — a tryptamine and the active metabolite of psilocybin — and 4-Iodo-2,5-dimethoxy-N-(2-methoxybenzyl) phenethylamine (25I-NBOMe) — a phenethylamine and new psychoactive substance — modulate extracellular neurotransmitter levels in the rat claustrum, as well as to examine their effects on wet dog shake behavior, a proxy for hallucinogenic activity.

METHOD(S): Microdialysis probes were stereotaxically implanted into the adult male Wistar Han rats' claustrum. Rats received local administration of either psilocin (100 or 500 µM) or 25I-NBOMe (500 µM) through the microdialysis probe. Dialysate samples were collected and analyzed using high-performance liquid chromatography (HPLC) with electrochemical detection to quantify extracellular levels of dopamine (DA), noradrenaline (NA), serotonin (5-HT), glutamate (GLU), γ-aminobutyric acid (GABA), and acetylcholine (ACh). A behavioral test defined as wet dog shakes (WDS) was conducted after drugs administration.

RESULTS: The obtained data revealed that both substances markedly altered extracellular levels of DA, 5-HT, GLU, GABA, and ACh. Psilocin significantly elevated also NA levels and produced the most pronounced enhancement of ACh signaling. 25I-NBOMe induced the greatest increase in 5-HT levels. Overall, psilocin generated a comparatively balanced excitatory–inhibitory neurochemical profile, reflecting its combined engagement of 5-HT2AR and 5-HT1AR, whereas 25I-NBOMe produced an excitation-biased pattern consistent with its selective, high-affinity 5-HT2AR agonism.

CONCLUSIONS: The observed complex neurotransmitter dynamics underscore the claustrum's role as an integrative hub, critical for mediating the effects of psychedelic compounds.

FINANCIAL SUPPORT: This research was funded by National Science Centre grant no. 2020/37/B/NZ7/03753 and statutory funds of the Maj Institute of Pharmacology, Polish Academy of Sciences.

## S3P17. LONG-TERM ADMINISTRATION OF LOW DOSES OF PSILOCYBIN CAUSES EPIGENETIC CHANGES IN THE RAT MEDIAL PREFRONTAL CORTEX

Agnieszka Wawrzczak-Bargieła, Małgorzata Jelonek-Kozioł, Weronika Kumorek, Korneliusz Banaś, Wiktor Bilecki, Marzena Maćkowiak

*Department of Pharmacology and Brain Biostructure, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Psychedelics have gained attention as potential treatments for depression, anxiety, PTSD, and substance use disorders. Compounds such as LSD, mescaline, ayahuasca, and psilocybin act mainly via 5-HT2A receptor activation, a key mediator of cortical signaling and neuroplasticity. A high-dose of psilocybin produces rapid antidepressant effects but may induce side effects. Low-dose of psilocybin "microdosing" may improve cognition and behavior while minimizing adverse effects, yet its molecular mechanisms remain unclear.

AIM(S): We examined whether psilocybin microdosing induces adaptive epigenetic changes in the rat medial prefrontal cortex (mPFC) of rats related to plasticity mechanisms.

METHOD(S): Male Wistar Han rats received psilocybin (0.05 mg/kg, s.c.) every 2 days for 21 days. Behavioral assessments included Acoustic Startle Response (ASR) and Novel Object Recognition (NOR) tests. Molecular analyses of the mPFC were performed 1 day or 8 days after the final dose, measuring total histone H3, acetylated H3K9/H3K27, BRD4/pBRD4, and HDAC2/HDAC4 using Western blot, PCR, and immunohistochemistry.

RESULTS: Psilocybin microdosing did not impair ASR or NOR and slightly increased novel object exploration,suggesting enhanced exploratory behavior.1 day post-treatment, H3K9ac and pBRD4 were elevated,with upregulation of Arc/Bdnf,indicating transient chromatin opening and increased transcription.Total BRD4 decreased,HDAC4 increased.By day 8, H3K9ac,pBRD4, and Arc/Bdnf normalized, whereas HDAC2 and total BRD4 remained reduced. 5-HT2A receptor levels were elevated, reflecting long-term transcriptional and serotonergic remodeling.

CONCLUSIONS: Repeated low-doses of psilocybin induces long lasting epigenetic adaptations without cognitive impairments. It triggers the two-phase molecular responses, with transient activation of plasticity genes (Arc/Bdnf) and enduring changes in HDAC2, BRD4, and 5-HT2A receptor, highlighting the role of epigenetic regulation in persistent cellular and serotonergic adaptations.

FINANCIAL SUPPORT: The research was funded by the National Science Center, Poland, grant 2024/08/B/NZ7/00930.

## S3P18. THE SCHIZOPHRENIA-ASSOCIATED 3Q29 DELETION MODIFIES BEHAVIORAL AND MOLECULAR RESPONSE TO ACUTE RISPERIDONE TREATMENT IN A MOUSE MODEL

Magdalena Ziemiańska[1], Klaudia Misiołek[1], Julia Netczuk[1], Martyna Boguska[1], Łukasz Szumiec[1], Sławomir Gołda[1], Mateusz Zięba[2], Marcin Piechota[2], Małgorzata Borczyk[2], Marzena Maćkowiak[3], Michał Korostyński[2], Jan Rodriguez Parkitna[1]

*[1] Departament of Molecular Neuropharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

*[2] Laboratory of Pharmacogenomics, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

*[3] Department of Pharmacology and Brain Biostructure, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Rare high-risk schizophrenia variants may contribute to variability in antipsychotic response, yet their mechanistic effects remain unclear. The 3q29 deletion, conferring >40-fold increased risk, is conserved in mice, providing a translational model to investigate disease mechanisms and treatment efficacy.

AIM(S): We investigated behavioral and transcriptomic responses to acute risperidone treatment in a mouse model of the human 3q29 deletion.

METHOD(S): Adult mutant and wild-type littermates were tested in two sessions, measuring acoustic startle and prepulse inhibition (PPI). Baseline responses were assessed in drug-naïve animals, followed by administration of risperidone (0.5 or 1 mg/kg) or saline in the second session. Brains were collected two hours after 1 mg/kg treatment for spatial transcriptomic profiling.

RESULTS: Mice carrying the 3q29 deletion exhibited elevated acoustic startle amplitudes while maintaining intact PPI, indicating increased baseline stimulus-evoked responding without impairment of sensorimotor gating. Acute risperidone treatment suppressed startle amplitude in both genotypes but produced a smaller reduction in PPI in 3q29 mice than in control mice, revealing blunted antipsychotic effects in mutants. At the molecular level, spatial transcriptomic analysis confirmed reduced expression of genes within the 3q29 deleted region. Following risperidone treatment, 64 genes showed genotype-dependent expression changes. Gene ontology analysis revealed enrichment for biological processes involving learning, synaptic plasticity, and sensorimotor function. Notably, 13 of these 64 genes are associated with schizophrenia risk.

CONCLUSIONS: Together, these findings demonstrate that genetic risk shapes behavioral and transcriptional responses to antipsychotic treatment. They validate the 3q29 mouse model for studying schizophrenia etiology and indicate that risperidone modulates genes implicated in disease risk.

FINANCIAL SUPPORT: This work was supported by National Science Centre, Poland NCN OPUS UMO-2020/39/B/NZ7/01494.

## S3P19. HBK-15 REDUCES MECHANICAL ALLODYNIA IN A MOUSE MODEL OF OXALIPLATIN-INDUCED NEUROPATHY

Barbara Mierzwiński[1], Selene Tertemiz[2], Aleksander Krzęstek[3], Klaudia Lustyk[4], Kinga Sałaciak[5], Karolina Pytka[6]

*[1] Laboratory of Experimental Neuropharmacology, Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University, Kraków, Poland*

*[2] Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Neuropathic pain remains a significant therapeutic challenge, affecting about 7-10% of the global population, with rising rates in cancer and diabetes. Chemotherapy-induced peripheral neuropathy (CIPN), especially from oxaliplatin, is hard to treat because current medications rarely fully relieve pain and often cause side effects.

AIM(S): HBK-15, a multimodal compound, has previously demonstrated antinociceptive effects in the formalin test and reduced allodynia in diabetic neuropathy; however, its effects in CIPN have not been investigated.

METHOD(S): Therefore, we aimed to evaluate the activity of HBK-15 in preventing the development of pain sensitization (mechanical allodynia and thermal

hyperalgesia) in an oxaliplatin-induced neuropathic pain model. Additionally, we examined potential mechanisms by assessing inflammatory and antioxidant-related markers in the spinal cord using qPCR for Nrf2 mRNA expression and Western blotting for IL-1β, TNF-α, Nrf2, and phosphorylated Nrf2 (pNrf2).

RESULTS: HBK-15 significantly reduced mechanical allodynia in the von Frey test after 7 days of treatment but had no effect during the acute phase of neuropathy. In contrast, the reduction of cold-induced thermal hyperalgesia was observed only in the early stage of symptom development at a dose of 5 mg/kg, with no effectiveness at later time points. Molecular analyses showed no significant changes in Nrf2 mRNA expression or in the protein levels of IL-1β, TNF-α, Nrf2, or pNrf2 following HBK-15 treatment.

CONCLUSIONS: These findings indicate that the analgesic effects of HBK-15 are likely not primarily mediated by inflammatory or Nrf2-related antioxidant pathways and may involve alternative mechanisms, possibly related to ion channel activity.

FINANCIAL SUPPORT: The study was financed by the Students' Scientific Society of JU MC within the Students' Grant Competition (2024/2025).

## S3P20. EVALUATION OF THE ANALGESIC EFFICACY, METABOLIC EFFECTS, AND IMPACT ON LOCOMOTOR ACTIVITY OF THE NOVEL HISTAMINE $H_3$ RECEPTOR ANTAGONIST LINS01022 IN A MURINE MODEL OF NEUROPATHIC PAIN

Magdalena Maciuszek[1], Mateusz Królewski[1], Magdalena Białoń[1], Nathalia A. Fiocchi[2], Flavia B. Lopes[2], João Paulo S. Fernandes[2], Katarzyna Popiołek-Barczyk[1]

[1] *Department of Neurochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Federal University of São Paulo, Department of Pharmaceutical Sciences, Diadema, Brazil*

INTRODUCTION: Neuropathic pain is a chronic condition that frequently shows limited responsiveness to conventional analgesics. Increasing evidence indicates that the histaminergic system may represent a promising target for pain management. In this study, we employed a novel compound, LINS01022 (1-(2,3-dihydrobenzofuran-2-yl)methylpiperazine), characterized as a potent H3R antagonist with nanomolar affinity (pKi=8.2).

AIM(S): The aim of this study was to evaluate the analgesic efficacy of the novel H3R antagonist LINS01022, as well as its effects on metabolic parameters and locomotor activity in a mouse model of neuropathic pain.

METHOD(S): Neuropathic pain was induced via chronic constriction injury (CCI) of the sciatic nerve. On day 14 post-injury, mice received a single intraperitoneal (i.p.) injection of LINS01022 at doses of 10, 20, or 30 mg/kg, while control animals were administered vehicle. Mechanical hypersensitivity was assessed using the von Frey test at 15, 45, 90, and 150 minutes following injection. Metabolic parameters as well as locomotor activity, were measured using the Promethion metabolic cage system. Mice subjected to CCI were administered LINS01022 (20 mg/kg) or vehicle and monitored in metabolic cages. Naïve animals served as healthy controls.

RESULTS: Our results demonstrated that LINS01022 exerted an analgesic effect at all tested doses compared to vehicle-treated controls. Importantly, its administration did not induce any significant alterations in metabolic parameters or locomotor activity.

CONCLUSIONS: Our findings provide the first evidence of the analgesic potential of this novel H3R antagonist. Moreover, the compound did not affect metabolic parameters or locomotor activity. Taken together, these results suggest that LINS01022 is a new promising agent with significant analgesic efficacy and a favorable safety profile and may serve as a valuable pharmacological tool for advancing our understanding of the histaminergic system in pathological conditions of the central nervous system.

FINANCIAL SUPPORT: Work was financed by a grant from the National Science Centre, Poland, SONATA 2019/35/D/NZ7/01042; and São Paulo Research Foundation – FAPESP grant 2023/03485-7 National Council for Scientific and Technological Development – CNPq grant 307829/2021-9.

## S3P21. DOES ORDER MATTER? TEMPORAL DYNAMICS OF SPATIAL SUMMATION OF PAIN

Daria Nowak[1], Tom Frankenstein[2], Tibor Szikszay[2], Kerstin Luedtke[2], Wacław M. Adamczyk[1,2]

[1] *Institute of Physiotherapy and Health Sciences, Academy of Physical Education, Katowice, Poland*

[2] *Institute of Health Sciences, Department of Physiotherapy, Pain & Exercise Research Luebeck (P.E.R.L.), University of Luebeck, Lübeck, German*

INTRODUCTION: Spatial summation of pain (SSp) occurs when perceived pain increases with the size of the stimulated area. The growth in pain intensity during SSp is often nonlinear and disproportionate relative to the extent of stimulation. However, the underlying mechanisms remain poorly understood.

AIM(S): This study aimed to investigate SSp in a temporally dynamic context, capturing its progression in real time.

METHOD(S): Electrical noxious stimulation was delivered via five electrodes attached to the foot, arranged in a continuous linear pattern either along or across the dorsum of the foot. Ten healthy participants (N=10) underwent four 25-second trials, each trial with a different electrode activation pattern: ascending (from one to five electrodes activated sequentially), descending (from five to one), random (random number of electrodes activated), and control (single, randomly selected electrode). Pain intensity was rated continuously using a computerised visual analogue scale (VAS).

RESULTS: Substantial SSp was observed, as indicated by a significant effect of the number of electrodes activated ($p<0.001$). However, the pattern of SSp varied depending on the type of trial ($p<0.001$). Ascending trials were more painful compared to descending trials ($p<0.001$) and control trials ($p<0.01$). Conversely, descending trials were less painful than random trials ($p<0.05$). Interestingly, random trials were equally painful as control trials, in which only a single electrode was activated.

CONCLUSIONS: This study demonstrates that a dynamic paradigm can effectively evoke spatial summation of pain and capture its temporal modulation. Moreover, the sequence of spatial input appears to influence pain perception independently of stimulus area, suggesting that the order of spatial recruitment may be a key factor in SSp mechanisms.

## S3P22. NEUROPROTECTIVE POTENTIAL AND BEHAVIORAL EFFICACY OF A NEW AGENT IN A MURINE MODEL OF NEUROPATHIC PAIN

Mateusz Królewski[1], Katarzyna Popiołek-Barczyk[1], Magdalena Białoń[1], Magdalena Maciuszek[1], Maciej Degutis[1], Marta Kędziora[1], Ryszard Bugno[2], Katarzyna Kaczorowska[2], Andrzej Bojarski[2], Katarzyna Starowicz[1]

[1] *Department of Neurochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Department of Chemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Neuropathic pain is a chronic condition typically resistant to conventional analgesics, including NSAIDs. This therapeutic resistance is driven by maladaptive neuroplasticity and glia-mediated chronic neuroinflammation, which perpetuate pain signaling independently of the initial peripheral insult. Although NSAIDs are not considered first-line treatments, they may be appropriate in select cases, particularly for patients with a mixed pain phenotype involving inflammatory mechanisms. Given this context, MIP001—an anti-inflammatory compound with a favorable safety profile—warrants further investigation as a potential therapeutic agent for neuropathic pain.

AIM(S): To determine the analgesic effects of MIP001 in a mouse model of neuropathic pain and to investigate its potential in vitro cytotoxicity and influence on glial-derived inflammatory mediators

METHOD(S): Neuropathic pain was induced in mice using the spared nerve injury (SNI) model. Mice received intraperitoneal (i.p.) MIP001 [20, 30 mg/kg], vehicle, or ibuprofen (30 mg/kg, positive control). Mechanical and thermal hypersensitivity were evaluated via von Frey and cold-plate tests, respectively. Additionally, in primary astroglial cultures, cytotoxicity (MTT test) and protein expression of inflammatory mediators (IL-1b, IL-6, IL-10, TNFα) were assessed following a 24h treatment with MIP001 [1, 10, and/or 50 μM] and/or lipopolysaccharide [100 ng/mL].

RESULTS: Our data showed that MIP001 dose- and time-dependently reduced mechanical and thermal hypersensitivity, providing significantly greater analgesia than ibuprofen. Furthermore, MIP001 did not alter protein expression or induce cytotoxicity in primary astrocytes, regardless of LPS treatment.

CONCLUSIONS: Our results showed that MIP001 has a significant analgesic effect on neuropathic pain, with an effect significantly greater than ibuprofen; however, the molecular mechanisms underlying this effect remain to be elucidated.

FINANCIAL SUPPORT: The study was supported by a programme coordinated by the Medical Research Agency, co-financed by the European Union under the NextGeneration EU initiative, within the framework of the National Recovery Plan, Component D, Investment D3.1.1 (project no. 2024/ABM/03/KPO/KPOD.07.07-IW.07-0173/24-00).

## S3P23. CELL-TYPE-SPECIFIC MYELINATION SHAPES WATER DIFFUSION ALONG THE TRIGEMINAL NERVE: EVIDENCE FROM ALONG-TRACT DTI PROFLING

Mateusz Orzełowski, Julia Krystowczyk, Łukasz Łabieniec
*Faculty of Physics, University of Bialystok, Białystok, Poland*

INTRODUCTION: Trigeminal neuralgia remains one of the most painful neurological conditions. While conventional imaging methods can show the structure of the trigeminal nerve, they provide limited insight into its microstructural integrity and functional status. To probe these subtle tissue properties the diffusion tensor imaging can be used. However, this method is still challenging for thin, cranial nerves.

AIM(S): The aim of this study was to reconstruct the trigeminal nerve along its full length using probabilistic method of axons tracking and to characterize how water diffusion directionality changes from the brainstem to Meckel's cave to explore whether this type of profiling could help to detect early microstructural changes in trigeminal nerve.

METHOD(S): Using probabilistic method of axons tracking, we reconstructed the trigeminal nerve in healthy volunteers and segmented it along the entire length into short parts from the brainstem to Meckel's cave. Then we measured water molecules diffusion directionality in each of these parts.

RESULTS: A downward trend in diffusion directionality was observed along the nerve's length, consistently with recent studies. This finding likely reflects the transition from central to peripheral myelination. Similar patterns have been suggested in recent studies on trigeminal neuralgia patients, supporting the idea that profiling of water diffusion directionality may detect early pathological changes.

CONCLUSIONS: These studies demonstrate that the diffusion signal is sensitive to the organization of myelin in nerves. As such, this effect should be carefully considered and validated before diffusion-based approaches are translated to the clinic.

FINANCIAL SUPPORT: Paid from funds of the Polish Ministry of Science and Higher Education under Agreement No. SKN/SP/630850/2025 of 11 April 2025, within the "Student Research Clubs Create Innovations 2024" programme.

## S3P24. HIERARCHICAL ORGANIZATION OF MECHANO-NOCICEPTIVE PATHWAYS REVEALED BY ACTIVITY LABELING

Gabriela Basile Carballo, Felipe Meira de Faria, Melisa Maidana Capitan, Ilona Szczot, Marek Brodzki, Christoffer Karlson, Leandro Flores do Nascimento, Håkan Olausson, Max Larsson, Marcin Szczot
*BKV/CSAN, Linköpings Universitetet, Linköping, Sweden*

INTRODUCTION: Nociception safeguards organisms from external injury and internal tissue damage by detecting and distinguishing harmful stimuli and encoding their intensity and location,thereby driving adaptive behavioral and homeostatic responses.However,the relationship between specific function and molecular identity across nociceptors remains poorly understood.

AIM(S): Here,we mapped the peripheral architecture of nociceptive signaling by combining in vivo activity labeling of pelvic nerve afferents with single-cell RNA sequencing to understand how different classes of nociceptors encode interoceptive signals

METHOD(S): To create a reference atlas,freshly dissociated cells in L5-S1 DRGs from adult C57BL/6Jrj mice were manually collected and sequenced using the Smart-Seq3 method.To label noxious stimuli-activated cells,we used animals that expressed the photoconvertible calcium reporter CaMPARI2.A series of nociceptive stimuli was delivered,while DRGs were exposed to conversion-inducing UV-light,fluorescently labeling activated neurons.Labelled cells were sequenced and embedded in the control cell atlas.

RESULTS: We developed a cell atlas with 1037 manually sorted neurons and detected 10,000 genes per cell.Sequencing of 2.997 labelled cells revealed that while nociceptive stimuli activated multiple neuronal classes,some were specific to visceral nociceptive signals.For C- nociceptors,Adra2a cells were abundant among colon-innervating neurons but nearly absent among skin-innervating populations.A-nociceptors showed clear target-specific specialization with Adm cells preferentially responding to colorectal stroke and anorectal distension.Moreover,we identified a subpopulation of Adm neurons,marked by the Uts2b+ gene,that relates to physiological bladder stimulation specifically.

CONCLUSIONS: These findings reveal a hierarchical organization of peripheral mechanical pain encoding,in which increasingly specialized mechano-nociceptor populations are differentially engaged according to tissue domain and organ context.

## S3P25. A COMPETITION MODEL FOR EXPRESSION OF NEURAL, PERCEPTION AND MOTOR SPONTANEOUS BEHAVIOR

Carlos M. Gómez

*Human Psychobiology Laboratory, University of Sevilla, Sevilla, Spain*

INTRODUCTION: A noninvasive alternative to the study of brain dynamics associated with behavior involves the systematic recording of external actions and the development of internal representational models that account for the observed patterns. Such modeling approaches can be constructed at varying levels of abstraction and complexity.

AIM(S): The present work evaluates the validity of a competition model (C-model) describing interactions among neural networks to explain alternating incompatible activities in different settings (mobility-immobility; saccadic-eye fixations; EEG microstates) and species(Drosophila malanogaster; humans).

METHOD(S): Data of mobility and immobility periods in Drosophila, eye fixation duration and EEG microstate durations in humans (data were extracted from open data bases), were analyzed following such model.

RESULTS: The frequency histograms of mobility-immobility in Drosophila, eye fixation duration and EEG microstate durations were fitted by the competition model. Others tested models as exgaussian and power law were also able to fit the data, with different outcomes for Akaike model comparisons.

CONCLUSIONS: The dynamics of neural networks controlling perceptions, actions and brain microstates, could be following a dynamics of competition between the different underlying networks, in order to keep a certain unity in brain microstates, actions and perceptions at any given moment.

FINANCIAL SUPPORT: (PID2022-139151OB-I00) Agencia española de investigación científica.

## S3P26. NMDA-INDUCED PLASTICITY OF TONIC GABAERGIC CURRENTS IN SST+ INTERNEURON SUBTYPES

Patrycja Droździel, Katarzyna Lebida, Jerzy W. Morzymas

*Department of Biophysics and Neuroscience, Wrocław Medical University, Wrocław, Poland*

INTRODUCTION: Effective neural signaling requires a balance of excitatory and inhibitory neurotransmission, both of which exhibit plasticity. GABAergic inhibition can be divided into synaptic (phasic) and extrasynaptic (tonic) components, each of which exhibits plastic properties. However, the mechanisms regulating tonic inhibition and its plasticity remain poorly understood.

AIM(S): We therefore investigated heterosynaptically induced plasticity of tonic currents in distinct subtypes of somatostatin-expressing interneurons (SST+IN).

METHOD(S): We studied CA1 hippocampal slices from SST-Cre × Ai14 mice. The tonic current density was determined from the current shift following picrotoxin application, normalized to the cell membrane capacitance. Cells were filled with biocytin and imaged by confocal microscopy to classify them as OLM-like or non-OLM. Using patch-clamp recordings and pharmacology, we measured tonic currents from SST+IN and induced heterosynaptic plasticity by a 3-minute NMDA (20μM) application.

RESULTS: Data show that non-OLM cells exhibited a lower tonic current density compared to OLM-like cells (p=0.016), which further decreased after plasticity induction with NMDA (p=0.005). On the contrary, OLM-like cells showed no significant changes after the same protocol. Next we examined α5-GABAAR contribution in the changes of tonic current density and found a significant increase in α5 subunit contribution in tonic current density in non-OLM cells after plasticity induction (p=0.009), whereas OLM-like cells showed no change. No differences in α5 involvement in OLM and non-OLM groups were detected under control conditions or after plasticity induction.

CONCLUSIONS: Our results indicate that OLM and non-OLM SST INs show substantially distinct tonic current densities. Moreover, only non-OLM INs show NMDA-induced plasticity which strongly depends on expression of α5 subunit containing GABAA receptors.

FINANCIAL SUPPORT: Supported by Polish National Science Centre grant OPUS 2021/43/B/NZ4/01675.

## S3P27. WORLD-FIXED *VERSUS* MONOCULAR EYE-FIXED DIRECTIONAL LIGHT BIASES ORIENTING AND INDUCES HEMISPHERIC ASYMMETRY OF LATERAL HABENULA ACTIVATION IN ALBINO RATS

Alicja Stankiewicz[1], Dominika Moskal[1], Martyna Marzec[1,2], Tomasz Błasiak[1]

[1] *Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*
[2] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Light can influence lateral habenula (LHb) activity via non-image-forming retinal pathways engaging retinorecipient thalamic nuclei. Because the LHb is an important regulator of midbrain dopaminergic system, hemispheric asymmetry within this pathway could translate into lateralized LHb recruitment, asymmetric dopaminergic output, and a directional bias in orienting behavior.

AIM(S): We tested whether world- *vs.* eye-centered directional light biases orienting and drives asymmetric LHb activation.

METHOD(S): Sprague–Dawley rats were tested in darkness (no ambient illumination) on an elevated platform that constrained locomotion to orienting movements. A directional LED light stimulus (10lx at animal's eye level; constant light; 15 minutes) was delivered in two reference frames: (i) world-centered, with the light source fixed relative to the platform, and (ii) eye-centered, with unilateral illumination aimed at the center of one eye, such that the stimulus remained stationary in the animal's visual field. Body orientation relative to the stimulus was quantified from overhead video. After the final session, brains were collected for anti c-Fos immunohistochemistry and LHb activation was compared between hemispheres.

RESULTS: During world-centered stimulation, rats typically rotate away from the light source, shifting body orientation to side-facing or back-facing relative to the stimulus direction rather than maintaining a forward-facing orientation toward the light. In the eye-centered condition, c-Fos labeling suggested hemispheric differences in LHb activation, with c-Fos signal concentrated in the medial LHb and showing interhemispheric asymmetry.

CONCLUSIONS: These findings show that world-fixed directional illumination biases body orientation relative to the light source. The interhemispheric asymmetry in LHb activation observed with unilateral eye-centered illumination raises the possibility that hemispheric imbalance in LHb recruitment contributes to orienting bias under asymmetric sensory input.

## S3P28. MAPPING THE RELAXIN-3/RXFP3 SYSTEM AND NUCLEUS INCERTUS CONNECTIVITY TO THE VENTROMEDIAL HYPOTHALAMUS: IMPLICATIONS FOR AGGRESSION-RELATED NETWORKS

Anna Janus[1,2,3], Sylwia Drabik[1,2], Kinga Przybylska[1,2], Gabriela Stopka[1,2], Gabriela Czerniak[1], Aleksandra Trenk[1], Anna Guguła[1], Anna Błasiak[1]

[1] *Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*
[2] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*
[3] *Sano Centre for Computational Medicine, Kraków, Poland*

INTRODUCTION: Violence is a profound global public health challenge a major global public health challenge, disproportionately affecting vulnerable populations. Within the psychosocial literature, violence is conceptualized as a specific manifestation of aggression. Aggression itself is an evolutionarily conserved social behaviour, however its underlying neural mechanisms remain understudied. Among the neural structures implicated in the regulation of aggressive behavior, the ventromedial hypothalamus (VMH) has consistently been shown to elicit aggressive responses upon stimulation. On the other hand, relaxin-3 (RLN3) and its receptor RXFP3 system has been implicated in the regulation of arousal and social anxiety–related behaviors, with evidence supporting its involvement in stress-induced social interactions and aggression-related responses. The principal source of RLN3 in the brain is the nucleus incertus (NI), a midline brainstem structure sensitive to stress-related cues.

AIM(S): We aimed to identify the anatomical circuitry through which RLN3/RXFP3 signaling may influence aggressive behavior.

METHOD(S): We employed neuronal tract tracing using an anterograde AAV2-mCherry viral vector, as well as immunohistochemical staining and in situ hybridization (RNAScope), to determine the anatomical connectivity between the NI and the VMH and to characterize its neurochemical profile.

RESULTS: Our results demonstrate that the VMH, particularly its ventrolateral subdivision, receives projections from the NI. Moreover, we show that the VMH contains RLN3-immunoreactive fibers and that VMH neurons express RLN3 receptors.

CONCLUSIONS: We provide evidence for an NI→VMHvl RLN3 pathway and RXFP3 expression in VMH neurons, supporting a potential role of RLN3/RXFP3 signaling in aggression-related VMH circuitry.

FINANCIAL SUPPORT: National Science Centre, Poland (UMO-2023/49/B/NZ4/01885).

## S3P29. GASOCRINE HYPOTHESIS: A POTENTIAL SUPPLEMENT TO CELL THEORY

Savani Anbalagan

*Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poznań, Poland*

INTRODUCTION: Oxygen is a major metabolic substrate and also a signaling molecule directly detected by oxygen gasoreceptors (eg., DosP phosphodiesterase in E. coli, FixL kinase in R. meliloti, GCY-35 soluble guanylate cyclase in C. elegans). But biology textbooks lack precise terms to describe oxygen-based inter-organismal signaling between oxygen-producing and aerobic organisms. To address this gap, I recently proposed the concept of gasocrine signaling, which encompasses all signaling mediated by gaseous molecules and gasoreceptors within and between cells, organisms, and even abiotic factors. Despite the fundamental importance of gaseous molecules for cells, cell theory lacks a gas-centric hypothesis.

AIM(S): The goal was to develop a new hypothesis about gases that would supplement the cell theory.

METHOD(S): Scientific literature on gas signaling and gas-sensing proteins in diverse organisms was read critically. Diverse proteins that are allosterically regulated and competitively inhibited by gas binding were considered potential gasoreceptors and proto-gasoreceptors, respectively.

RESULTS: Proteins such as hemoglobin were proposed as oxygen or carbon dioxide gasoreceptors. To date, no gas-sensing nucleic acids have been reported; hence, the concept of gas-sensing riboceptors was proposed. A gasocrine hypothesis—that all cells require gasocrine signaling—was proposed. Consciousness has also been proposed as a sum of gasocrine signaling. A perspective manuscript was published in the Polish Biochemical Society journal – Acta Biochimica Polonica (https://doi.org/10.3389/abp.2025.15465).

CONCLUSIONS: If the Gasocrine Hypothesis withstands falsification, it will supplement cell theory by providing a novel framework for understanding the role of gases and establish one of the foundations of the emerging field of Gasocrinology. It also holds relevance for neurodevelopmental disorders and neurodegenerative diseases.

## S3P30. CIRCUMIACENTIUM HYPOTHESIS: A POTENTIAL SUPPLEMENT TO CELL THEORY

Savani Anbalagan

*Institute of Molecular Biology & Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland*

INTRODUCTION: Environmental factors play a major role in neurodevelopment and diseases. Numerous classes of receptors sense environmental factors and trigger cellular signaling in diverse cellular and anatomical regions. Despite the fundamental importance of environment factors for cells, cell theory lacks an environment-centric hypothesis.

AIM(S): The goal was to develop a new hypothesis about the environment that would supplement the cell theory.

METHOD(S): Scientific literature on the role of environmental factors in diverse organisms was read critically. Diverse proteins—allosterically regulated ones as potential receptors, competitively inhibited ones as proto-receptors—were considered responsive to environmental factors.

RESULTS: A Circumiacentium hypothesis—that all cells are limited by their environment—was proposed. Several potential experiments to falsify this hypothesis were also proposed.

CONCLUSIONS: If the Circumiacentium hypothesis withstands falsification, it will supplement cell theory by providing a novel framework for understanding the environment's role in cells. It also holds relevance for neurodevelopmental disorders and neurodegenerative diseases.

## S3P31. ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF 5-HT DORSAL RAPHE NEURONS IN WISTAR KYOTO RATS

Agnieszka Kania, Marcin Siwiec, Agata Krużel, Michał Kiełbiński, Krzysztof Tokarski

*Department of Physiology, Maj Institute of Pharmacology Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Major depressive disorder (MDD) is one of the main causes of disability worldwide, with approximately 30% of patients failing to respond to standard treatment methods, such as selective serotonin reuptake inhibitors (SSRI). The term treatment-resistant depression (TRD) was coined to describe depressive symptoms that fail to improve after at least two courses of adequate treatment. Although knowledge about this disorder is limited, there are animal models that are used to study its etiology. Wistar Kyoto rats display a stress-prone and depression-like phenotype, although little is known about its underlying morphological and neurochemical substrates, especially in the dorsal raphe (DRN) – the main serotoninergic (5-HT) nucleus in the brain. This animal model responds to ketamine, but not SSRI treatment, which might indicate altered serotonin system function and/or morphology.

AIM(S): We aimed to morphologically and electrophysiologically characterize 5-HT DRN neurons in Wistar and Wistar Kyoto rats to gain insight about the state of the serotonin system in the TRD model.

METHOD(S): We performed whole-cell patch clamp recordings of intrinsic passive and active membrane properties, as well as excitatory and inhibitory transmission in the DRN. All cells were filled with biocytin during recording and slices were later immunostained for tyrosine- and tryptophan hydroxylase, as markers of dopaminergic and serotonergic neurons respectively.

RESULTS: Preliminary recordings have revealed a heterogenous distribution of morphological and electrophysiological features of 5-HT DRN neurons in both strains. Moreover, these characteristics correlate to anatomical subdivisions within the broad DRN hub.

CONCLUSIONS: As 5-HT remains one of the main neuromodulators involved in mood regulation, the differences we observed might contribute towards understanding the distinct neural substrates of the behavioral characteristics and treatment-resistant phenotype observed in Wistar Kyoto rats.

FINANCIAL SUPPORT: Grant NCN OPUS 25 nr.: 2023/49/B/NZ4/03592 ID: 591148.

## S3P32. ELECTROPHYSIOLOGICAL CHARACTERISTICS OF DENTATE GYRUS GRANULE CELLS IN WISTAR KYOTO RATS

Martyna Bernaciak[1,2], Marcin Siwiec[2], Krzysztof Tokarski[2]

[1] *Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

[2] *Department of Physiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Dentate gyrus(DG), positioned between the entorhinal cortex and CA3 area of the hippocampus, is critical for learning, memory, spatial navigation, and mood regulation. Decreased DG neurogenesis is hypothesized to be an underlying factor of depression. Additionally, deficits in DG granule cells(DGCs) have been shown to contribute to the development of various psychiatric disorders, including major depressive disorder(MDD). Wistar Kyoto(WKY) rats show intrinsic depression traits, including despair-like behavior, passive coping with stress as well as exaggerated physiological response to stressful environment.

AIM(S): We aimed to electrophysiologically characterize dentate gyrus granule cells in Wistar and Wistar Kyoto rats.

METHOD(S): We performed whole-cell patch clamp recordings of DG granule cells, focusing on their intrinsic passive membrane properties. Neurons were filled with biocytin during recording and slices were later treated with streptavidin-conjugated fluorophores to confirm neuronal phenotype.

RESULTS: Preliminary recordings have revealed a heterogenous distribution of electrophysiological features of DG granule cells in both strains.

CONCLUSIONS: This characterization might contribute towards understanding the distinct neural substrates of the behavioral characteristics and treatment-resistant phenotype observed in Wistar Kyoto rats. These findings highlight the DG as a potential target for understanding the cellular basis of depressive-like traits.

## S3P33. ACTION POTENTIAL PROPERTIES OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONS IN XKR8 KNOCKOUT MICE DURING POSTNATAL DEVELOPMENT

Igor Nagula[1], Emilija Kavalnyte[1], Kornelija Vitkute[1], Daiva Dabkeviciene[3], Urtė Neniškytė[1,2], Aidas Alaburda[1]

[1] *Department of Neurobiology and Biophysics, Vilnius University Life Sciences Center, Vilnius, Lithuania*

[2] *Vilnius University Life Sciences Center, VU-EMBL Partnership Institute, Vilnius, Lithuania*

[3] *National Cancer Institute, Vilnius, Lithuania*

INTRODUCTION: The first three postnatal weeks are characterized by morphological and electrophysiological maturation of hippocampal neurons, driven by the incorporation of ion channels and receptors and coinciding with peak synaptogenesis. Phosphatidylserine, a neuronal "eat me" signal translocated by the Xkr8 scramblase, is one of the signals that regulate synaptic pruning. The Xkr8 scramblase is highly expressed in mouse brains after birth, and its deficiency leads to excessive excitatory nerve terminals, altered neuronal bouton morphology, and increased cortico-cortical and cortico-spinal projections. Although altered synaptic activity has been reported in Xkr8-deficient mice, its impact on intrinsic membrane properties has not been systematically investigated.

AIM(S): Here, we investigated action potential (AP) properties of hippocampal CA1 pyramidal neurons in Xkr8 knockout and wild-type mice across postnatal development in males and females

METHOD(S): Whole-cell patch-clamp recordings were performed in acute mouse brain slices, and AP amplitude, width, and threshold were analyzed.

RESULTS: Three-way ANOVA (Age × Genotype × Sex) revealed a significant main effect of age on AP amplitude, width, and threshold ($P<0.001$). Importantly, significant Age × Genotype interactions were detected for all these properties ($P\leq0.01$), indicating genotype-dependent alterations in developmental trajectories. No genotype-related interactions with sex were observed, although a main effect of sex was detected for AP width and threshold.

CONCLUSIONS: Together, these findings indicate that Xkr8 deficiency modifies the maturation pattern of intrinsic membrane properties across postnatal development.

## S3P34. SUBCHRONIC SEMAGLUTIDE ADMINISTRATION PERTURBS LONG-TERM POTENTIATION IN THE MEDIAL PREFRONTAL CORTEX IN MICE

Karolina Różewicz[1,2], Bartosz Bobula[2], Agnieszka Kania[2], Krzysztof Tokarski[2]

[1] *Institute of Biology and Earth Sciences, University of the National Education Commission, Kraków, Poland*

[2] *Departament of Physiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Glucagon-like peptide-1 receptor agonists (GLP-1RAs), such as semaglutide, are widely used in the treatment of type 2 diabetes and obesity, with an increasing prevalence of off-label use. In addition to their metabolic effects, GLP-1RAs affect the function of the central nervous system. Emotional regulation, cognitive processes and reward-related behaviour are all significant functions of the medial prefrontal cortex (mPFC). However, semaglutide's impact on synaptic plasticity in this region of the brain remains uncertain.

AIM(S): To evaluate the effects of semaglutide on synaptic transmission and plasticity in the medial prefrontal cortex in mice.

METHOD(S): Semaglutide (3 nmol/kg) or physiological saline was administered to C57BL/6 mice on a daily basis for a period of 10 days. Electrophysiological recordings were conducted in acute mPFC slices subsequent to treatment. Paired-pulse facilitation (PPF), long-term potentiation (LTP) and basal synaptic transmission were evaluated. The experiment included the monitoring of body weight.

RESULTS: No significant changes were observed in baseline synaptic transmission or PPF between groups. However, the mPFC of semaglutide-treated mice exhibited a substantial decrease in LTP induction compared to controls, suggesting that synaptic plasticity was compromised. Additionally, semaglutide administration led to reduced body weight gain.

CONCLUSIONS: Semaglutide disrupts LTP induction in the medial prefrontal cortex, which implies that it may have an impact on reward-related circuitry and synaptic adaptability. These findings highlight the need for further research into the long-term neurophysiological effects and safety profile of GLP-1RAs within the central nervous system, particularly given their expanding clinical and off-label use.

## S3P35. HIDDEN IN THE BRAINSTEM: NUCLEUS INCERTUS AS A NEW REGULATOR OF SOCIOSEXUAL BEHAVIOUR

Gabriela Czerniak[1], Gabriela Stopka[1,2], Kinga Przybylska[1,2], Sylwia Drabik[1,2], Patryk Sambak[1,2], Anna Błasiak[1]

*[1] Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*
*[2] Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: The brainstem nucleus incertus (NI) plays a key regulatory role in the control of stress, anxiety, and social interactions. It is the primary source of the neuropeptide relaxin-3 (RLN3), which signals through its receptor RXFP3. The NI provides dense RLN3ergic projections to the forebrain bed nucleus of the stria terminalis (BNST), a region critically involved in the control of anxiety, stress, and social and sexual behaviours. Consistent with this connectivity, the BNST exhibits high RXFP3 expression. However, the molecular characteristics of the NI-BNST axis, particularly concerning the RLN3/RXFP3 systems and its contribution to sociosexual behaviour, remain poorly understood.

AIM(S): This study aimed to investigate the involvement of the NI in sociosexual behaviour in male and female Sprague–Dawley rats. Specifically, we aimed to: (i) assess changes in NI neuronal activity associated with sociosexual behaviour; (ii) characterise the distribution and origin of RLN3-positive fibres in the BNST; and (iii) evaluate the direct sensitivity of BNST neurons to RLN3 administration.

METHOD(S): The involvement of NI in sociosexual behaviour was assessed using c-Fos immunohistochemistry. The source of RLN3-ergic fibres innervating the BNST was identified using viral anterograde tract tracing. Ex vivo patch-clamp recordings were performed in BNST neurons to evaluate their sensitivity to RLN3.

RESULTS: Male rats that interacted with females showed a higher density of c-Fos–positive cells in the NI compared to control males, whereas no such difference was observed in females. RLN3-positive fibres originating from the NI were identified in the BNST. During patch-clamp recordings, RLN3 application elicited a whole-cell outward current in BNST neurons.

CONCLUSIONS: These findings position the NI as an important brainstem contributor to male sociosexual behaviour and identify the BNST as a functional target of RLN3/RXFP3 signalling.

FINANCIAL SUPPORT: National_Science_Centre, Poland: UMO-2023/49/B/NZ4/01885.

## S3P36. PATCH-CLAMP RECORDINGS FROM RETROGRADELY LABELED SPINAL PROJECTION NEURONS IN THE RAT

Agata Krużel, Marcin Siwiec, Michał Kiełbiński

*Department of Physiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Pain is a highly prevalent and debilitating condition affecting a large proportion of the human population, highlighting the need for improved understanding of the neural circuits underlying nociceptive processing. Importantly, not all retrograde tracers provide sufficient signal quality for targeted electrophysiological recordings.

AIM(S): Here, we aimed to evaluate different retrograde labeling approaches to enable patch-clamp recordings from anatomically defined spinal projection neurons.

METHOD(S): We compared several tracers (fluorescent pink and green dyes, and Alexa Fluor 488–conjugated cholera toxin) in adult rats. We stereotaxically injected 100 nl volumes of these compounds into the brainstem nucleus lateralis parabrachialis (coordinates: AP −8.9; ML ±2.1; DV −6.8 from bregma) in adult (10-week-old) Wistar rats of both sexes. Surgery was performed under general anesthesia and followed by a recovery period of 10–14 days, after which animals were either perfused for fixation and imaging or killed by decapitation for patch-clamp electrophysiological recordings.

RESULTS: We observed efficient retrograde tracing from the brainstem injection site, with numerous labeled spinal neurons located in laminae I–V and X at 12–14 days post-injection. Furthermore, we successfully obtained patch-clamp recordings from these neurons and determined that red-shifted (pink) fluorescent dyes are the most suitable for this purpose, most likely due to reduced light scattering.

CONCLUSIONS: Red-shifted (pink) fluorescent dyes enable reliable retrograde labeling of spinal projection neurons from the nucleus lateralis parabrachialis and are optimal for targeted patch-clamp recordings. This approach provides a robust tool for studying defined nociceptive circuits.

FINANCIAL SUPPORT: This research was supported by the National Center for Science grant nr 2024/08/X/NZ4/00622 awarded to MK.

## S3P37. A HIGH-DENSITY ELECTROPHYSIOLOGICAL FRAMEWORK FOR POPULATION-LEVEL CHARACTERIZATION OFD SPINAL SENSORY NEURONS *IN VIVO*

Joanna Bernacka[1], Eliza Kramarska[2], Mateusz W. Kucharczyk[1,2,3]

[1] *Cancer Neurophysiology Research Group, Polish Centre for Technology Development, Łukasiewicz-PORT, Wrocław, Poland*

[2] *Biophotonics and Electrophysiology Lab, Bioimaging Laboratories, Center for the Development of Therapies for Civilization and Age-Related Diseases, Jagiellonian University Medical College, Kraków, Poland*

[3] *Wolfson Sensory, Pain and Regeneration Centre, King's College London, United Kingdom*

INTRODUCTION: Spinal dorsal horn is the first central stage of somatosensory integration, where peripheral inputs and intensity are transformed into ascending signals. While individual spinal neurons have been extensively studied, the population-level encoding of sensory information across dorsal horn laminae remains less studied. Single-unit recordings limit insight into how spinal neurons collectively represent stimulus features. We decided to use high-density electrophysiological recordings for sampling of large neuronal ensembles, enabling investigation of spinal sensory processing at the population level.

AIM(S): To establish a high-density in vivo framework for investigating population-level sensory encoding in the mouse spinal dorsal horn.

METHOD(S): We performed an acute in vivo recording accross the lumbar spinal cord of anesthetized mice using a Neuropixels 1.0 probe. Graded mechanical, light tactile, cold, and electrical stimulation was presented to the receptive fields on animal's ipsilateral hind paw. Neuronal depth was reconstructed from the probe geometry. Spike sorting was performed using Kilosort4 with manual curation in Phy. Downstream analyses were implemented in custom Python pipelines.

RESULTS: This framework enabled characterization of spinal neurons based on multimodal responsiveness, graded intensity coding, and laminar localization. Population level recordings reveal coordinated activity patterns across dorsal horn layers, suggesting structured sensory representations rather than isolated unit responses. This approach enables identification of neuron subtypes with distinct responses to different stimuli and spinal locations.

CONCLUSIONS: High-density population recordings provide a scalable method for investigating spinal sensory coding beyond single-neuron descriptions. By integrating multimodal stimulation with laminar and population-level analysis, this paradigm advances the study of spinal circuit organization and the network mechanisms underlying sensory integration.

FINANCIAL SUPPORT: Funded by National Science Centre grant (2022/D/NZ4/02676), held by MW Kucharczyk.

## S3P38. THE ROLE OF 5-HT7 RECEPTOR ACTIVATION IN HIPPOCAMPAL SYNAPTIC PLASTICITY IN ADULT AND JUVENILE MICE

Nikola Multan[1], Bartosz Bobula[1], Monika Bijata[2], Krzysztof Tokarski[1]

[1] *Department of Physiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Laboratory of Cell Biophysics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Previous studies indicate that the 5-HT7 receptor may play a crucial role in the modulation of neuroplasticity. Activation of this receptor has been shown to rescue hippocampal long-term potentiation (LTP) in brain slices obtained from rodent models of nervous system disorders and to influence neuronal cytoarchitecture under physiological conditions. Moreover, mice lacking the 5-HT7 receptor exhibit impaired hippocampal LTP and deficits in contextual learning. Given that earlier studies indicate higher expression of the 5-HT7 receptor during early postnatal development, we examined brain slices obtained from both adult and juvenile mice.

AIM(S): We investigated the effects of 5-HT7 receptor activation on synaptic plasticity in hippocampal slices obtained from healthy adult (8-12 weeks old) and juvenile (13-15 postnatal days) mice.

METHOD(S): Extracellular recordings of field excitatory postsynaptic potentials (fEPSPs) were performed in the CA1 region. The 5-HT7 receptor was activated by bath application of the selective agonist LP-211. LTP was induced using theta-burst stimulation (TBS). In addition, paired-pulse stimulation was applied to assess short-term synaptic plasticity.

RESULTS: In contrast to previous reports suggesting a role in plasticity modulation, activation of the 5-HT7 receptor did not affect either long-term or short-term synaptic plasticity in the CA1 region of adult mice. Similarly, no significant differences were observed between control slices and those treated with LP-211 in juvenile mice.

CONCLUSIONS: These findings suggest that activation of the 5-HT7 receptor does not modulate synaptic plasticity in the CA1 region of either adult or juvenile mice under physiological conditions. It is possible that the functional role of the 5-HT7 receptor becomes more important under pathological conditions.

FINANCIAL SUPPORT: Nr rejestracyjny: 2019/35/D/NZ4/02042; Kierownik projektu: dr Monika Bijata.

## S3P39. ENDOCANNABINOIDS FACILITATE LONG-TERM POTENTIATION (LTP) OF EXCITATORY INPUTS ONTO VASOACTIVE INTESTINAL POLYPEPTIDE-EXPRESSING INTERNEURONS (VIP-INS) IN LAYER 2/3 OF THE MOUSE NEOCORTEX

Karolina Bogaj, Joanna Urban-Ciećko

*Laboratory of Electrophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Learning process occurs through synaptic plasticity. Cellular model of synaptic plasticity is called long-term potentiation (LTP), in which specific stimulation of inputs results in long-lasting strengthening of the synaptic transmission. In the present study, we described LTP at excitatory inputs onto layer 2/3 vasoactive intestinal polypeptide-expressing interneurons (VIP-INs) in the mouse somatosensory cortex. VIP-INs contribute to one of three main inhibitory neuron classes. However, VIP-INs possess a unique function within the neuronal network, because they promote excitation by targeting and inhibiting other populations of GABAergic cells and thus disinhibiting glutamatergic neurons.

AIM(S): Our study aims to unravel the mechanism of LTP onto cortical VIP-INs.

METHOD(S): We studied fluorescently-labelled VIP-INs in acute brain slices of transgenic mice, utilizing whole-cell patch clamp technique. LTP was evoked with a pairing paradigm, where extracellular stimulation was paired with postsynaptic depolarization of the membrane.

RESULTS: Pharmacological blockage of specific receptors revealed that LTP at excitatory synapses on layer 2/3 VIP-INs is NMDAR- and mGluR-5-independent. In contrast, LTP is controlled by mGluR-1 and L-type voltage-gated calcium channels. Furthermore, Src-family kinase signaling contributes to this process, however without involvement of common pathway through transient receptor potential channels (TRPC). Paired-pulse ratio and coefficient of variation analyses hint toward presynaptic mechanism of LTP expression. Looking for a retrograde messenger that mediates LTP expression, we found that postsynaptically synthesized endocannabinoids, but not brain-derived neurotrophic factor (BDNF), are involved in LTP at VIP-INs.

CONCLUSIONS: Altogether, our study describes for the first time mechanism of LTP at excitatory inputs onto layer 2/3 VIP-INs, showing endocannabinoid signaling as an unconventional retrograde factor in LTP expression.

FINANCIAL SUPPORT: This research was funded by the National Science Centre, Poland, OPUS grant no. 2020/39/B/NZ4/01462 to JUC.

## S3P40. EFFECT OF SSR504734, A SELECTIVE GLYCINE TRANSPORTER TYPE 1, IN THE AMYGDALA KINDLING MODEL OF TEMPORAL LOBE EPILEPSY IN MICE

Nikola Gapińska[1,2], Ewelina Mitura[1], Piotr Wlaź[1], Krzysztof Kamiński[3], Marcin Jakubiec[3], Michał Abram[3], Bernadeta Szewczyk[4], Anna Rafało-Ulińska[4], Katarzyna Ciepiela[5], Weronika Woźniak[1,2], Agata Matuła[1,2], Katarzyna Socała[1]

[1] *Biomedical Research Laboratory, Faculty of Biology and Biotechnology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland*

[2] *Doctoral School of Quantitative and Natural Sciences, Maria Curie-Skłodowska University, Lublin, Poland*

[3] *Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*

[4] *Department of Neurobiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[5] *Selvita S.A., Kraków, Poland*

INTRODUCTION: Glycine transporter type 1 (GlyT1) plays a crucial role in regulating extracellular glycine concentrations by maintaining the balance between excitatory and inhibitory neurotransmission in the central nervous system. Preclinical evidence indicates that selective inhibition of GlyT1 can modulate seizure susceptibility and influence hippocampal network activity, suggesting a potential role of this transporter in epileptogenesis.

AIM(S): Here, we aimed to investigate the effect of SSR504734, a selective GlyT1 inhibitor, on the progression of epileptogenesis in the amygdala kindling model of temporal lobe epilepsy in mice. Potential behavioral alterations were also assessed, including motor coordination and learning and memory.

METHOD(S): CD-1 male mice were divided into four experimental group: kindled and sham (non-kindled)

mice receiving SSR504734 at 30 mg/kg or saline daily throughout the amygdala kindling procedure. Mice were considered fully-kindled until five consecutive generalized seizures were elicited according to Racine scale. To evaluate potential behavioral alterations mice were tested in a two of assays, i.e. spatial memory was assessed in the Morris water maze test and motor coordination in the rotarod test.

RESULTS: SSR504734 did not inhibit the progression of epileptogenesis in kindled mice. Also, no significant differences were observed between kindled and sham mice in any of the behavioral tests.

CONCLUSIONS: The obtained results suggest that SSR504734 does not modulate progression of epileptogenesis in the amygdala kindling model. Furthermore, under the conditions tested, kindling did not induce alterations in motor or cognitive behaviors in mice.

FINANCIAL SUPPORT: Research project financed by the National Science Center UMO-2021/41/B/NZ7/00328.

## S3P41. THERAPEUTIC EFFECT OF LOW-INTENSITY PULSED ULTRASOUND ON COMORBID ANXIETY AND DEPRESSION IN PTZ-KINDLED RATS

Mykhailo Pervak[1], Olha Yehorenko[1], Kseniia Prybolovets[2], Natalia Bukreeva[3], Olha Kashchenko[2], Svitlana Liashenko[2]

[1] *Department of Simulation Medical Technologies, Odesa National Medical University, Odesa, Ukraine*

[2] *Department of Physiology, Pathological Physiology, Medical Physics and Informatics, Odesa National Medical University, Odesa, Ukraine*

[3] *Molecular and Genetic Laboratory, Odesa National Medical University, Odesa, Ukraine*

INTRODUCTION: Epilepsy is frequently accompanied by comorbid anxiety and depression. Transcranial Low-Intensity Pulsed Ultrasound (LIPUS) is a promising non-invasive neuromodulatory technique capable of restoring neuronal activity and exerting anti-inflammatory effects.

AIM(S): To evaluate the efficacy of LIPUS stimulation (1 *vs.* 5 sessions) on anxiety- and depressive-like behaviors in Wistar rats with pentylenetetrazol (PTZ)-induced kindling.

METHOD(S): The study involved 42 sexually mature Wistar rats. Chronic epileptic syndrome was modeled by daily intraperitoneal injections of PTZ (35 mg/kg) for 21 days. LIPUS stimulation (frequency 1.5 MHz, intensity 30 mW/cm2, pulse duration 200 μs) was applied to the parieto-occipital areas (20 min/day). Rats were divided into intact and kindled groups receiving either sham or active LIPUS (1 or 5 sessions). Anxiety levels were assessed using the Open Field (OF) test, and depressive-like behavior was evaluated using the Porsolt Forced Swim Test (FST). Data were analyzed using ANOVA with Tukey's post-hoc test.

RESULTS: Kindled rats exhibited significant behavioral deficits compared to controls, characterized by reduced locomotor activity and increased anxiety in the OF test, as well as increased immobility in the FST. A single LIPUS session did not yield significant therapeutic effects. However, a course of five LIPUS sessions significantly restored behavioral parameters. In the OF test, the total number of crossed squares increased by 38.5% ($P<0.01$) and central square entries increased by 65.3% compared to the sham-treated kindled group. In the FST, the duration of immobility decreased by 23.5% ($P<0.01$), indicating a reduction in depressive-like behavior.

CONCLUSIONS: A five-session course of transcranial LIPUS effectively mitigates behavioral comorbidities in a rat model of PTZ-induced epilepsy. These findings suggest that LIPUS may serve as a potential non-pharmacological strategy for managing anxiety and depression associated with epilepsy.

FINANCIAL SUPPORT: None declared.

## S3P42. SYNERGISTIC EFFECTS OF RAPAMYCIN AND PIOGLITAZONE ON METABOLIC AND SYSTEMIC COMORBIDITIES IN THE PTZ-KINDLING MODEL OF EPILEPSY

Mykhailo Pervak[1], Kseniia Prybolovets[2], Leonid Godlevsky[2], Natalya Bukreeva[3], Serhii Marchenko[2]

[1] *Department of Simulation Medical Technologies, Odesa National Medical University, Odesa, Ukraine*

[2] *Department of Physiology, Pathological Physiology, Medical Physics and Informatics, Odesa National Medical University, Odesa, Ukraine*

[3] *Molecular and Genetic Laboratory, Odesa National Medical University, Odesa, Ukraine*

INTRODUCTION: Glucose dysregulation increases seizure susceptibility and underlies systemic complications in epilepsy.

AIM(S): This study evaluated common pathogenesis markers in a PTZ-induced kindling model and assessed the therapeutic potential of targeting mTOR and PPAR-γ pathways.

METHOD(S): Wistar rats underwent a 21-day PTZ-kindling protocol. Treatment groups received daily administration of Rapamycin (mTOR inhibitor), Pioglitazone (PPAR-γ agonist), or a combination of both, starting after the 11th PTZ injection. Seizure severity was assessed daily. Post-experimental analysis included biochemical screening (glucose, insulin, HOMA-IR), brain TRPV1 content measurement, and histomorphological evaluation of myocardial and liver tissues.

RESULTS: Combined therapy protected 5/7 rats from generalized seizures. Kindled rats showed hyperglycemia (+28.6%), hyperinsulinemia (+47.0%), and a 62.0% increase in HOMA-IR compared to controls. Combined treatment reversed these metabolic shifts, whereas Rapamycin alone was ineffective. Brain TRPV1 levels rose by 40.5% in kindled rats but decreased by 38.0% after combined therapy. Furthermore, combined treatment prevented systemic damage, including myocardial microhemorrhages, cardiomyocyte thickening, and perivenular liver fibrosis with Kupffer cell infiltration, which were observed in untreated kindled rats.

CONCLUSIONS: Our findings highlight that chronic kindling induces significant systemic impairments, including hyperglycemia, hyperinsulinemia, and peripheral organ damage (heart and liver). A short-term course of pioglitazone, intensified by rapamycin, not only reduces seizure severity but also mitigates these diabetes-related manifestations. This suggests that targeting metabolic pathways and transient receptor potential channels (TRPV1) may offer a novel multi-target strategy for managing epileptic comorbidities.

FINANCIAL SUPPORT: Supported by the internal research budget of Odesa National Medical University.

## S3P43. THE EFFECT OF SSR504734, A GLYCINE TRANSPORTER TYPE 1 INHIBITOR, ON ANXIETY RELATED BEHAVIOR IN A MOUSE MODEL OF TEMPORAL LOBE EPILEPSY

Weronika Woźniak[1,2], Nikola Gapińska[1,2], Piotr Wlaź[1], Krzysztof Kamiński[3], Marcin Jakubiec[3], Michał Abram[3], Katarzyna Ciepiela[4], Agata Matuła[1,2], Katarzyna Socała[1]

*[1] Biomedical Research Laboratory, Faculty of Biology and Biotechnology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland*
*[2] Doctoral School of Quantitative and Natural Sciences, Maria Curie-Skłodowska University, Lublin, Poland*
*[3] Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*
*[4] Selvita S.A., Kraków, Poland*

INTRODUCTION: Background: Epilepsy affects approximately 1% of the global population and, despite significant advances in research, remains a major therapeutic challenge. In addition to recurrent seizures, patients with epilepsy frequently experience a substantial burden of comorbidities, particularly psychiatric disorders, including anxiety. Preclinical studies suggest that selective inhibition of the glycine transporter GlyT1 may influence susceptibility to epileptic seizures.

AIM(S): The aim of the present study was to evaluate the effect of the GlyT1 inhibitor SSR504734 on anxiety-related behavior in mice subjected to amygdala kindling, an experimental model of temporal lobe epilepsy.

METHOD(S): Methods: The experiments were conducted on male CD-1 mice divided into four groups: kindled and sham (non-kindled) animals. Mice received SSR504734 at a dose of 30 mg/kg or physiological saline daily throughout the stimulation period. Seizure severity was assessed using the Racine scale, and animals were considered kindled after five consecutive generalized seizures. Anxiety-related behaviors were evaluated by using the elevated plus maze and the light/dark box tests. In addition, general locomotor activity was measured to assess the effect of treatments on motor performance.

RESULTS: Results: No effects were observed on spontaneous activity and anxiety behaviour in the light dark box test. However, mice treated with SSR504734 spent significantly more time in the open arms in the elevated plus maze test. In addition, amygdala stimulation was associated with behavioral changes compared with sham groups.

CONCLUSIONS: Conclusion: The elevated plus maze results indicate that SSR504734 may reduce anxiety-like behavior, suggesting a potential anxiolytic effect of this compound.

FINANCIAL SUPPORT: Research project financed by the National Science Center UMO 2021/41/B/NZ7/00328.

## S3P44. EFFECT OF SSR504734, A SELECTIVE GLYCINE TRANSPORTER TYPE 1 INHIBITOR, ON EPILEPTOGENESIS AND DNA METHYLATION STATUS IN THE AMYGDALA KINDLING MODEL IN MICE

Agata Matuła[1,2], Nikola Gapińska[1,2], Piotr Wlaź[1], Anna Rafało-Ulińska[4], Bernadeta Szewczyk[4], Krzysztof Kamiński[4], Marcin Jakubiec[4], Michał Abram[4], Katarzyna Ciepiela[5], Weronika Woźniak[1,2], Katarzyna Socała[1]

[1] *Biomedical Research Laboratory, Institute of Biological Sciences, Maria Curie-Skłodowska University, Lublin, Poland*
[2] *Doctoral School of Quantitative and Natural Sciences, Maria Curie-Skłodowska University, Lublin, Poland*
[3] *Department of Neurobiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*
[4] *Department of Medicinal Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland*
[5] *Selvita S.A., Kraków, Poland*

INTRODUCTION: Glycine is a unique neurotransmitter in the central nervous system that modulates both glycinergic and glutamatergic transmission. Through glycine reuptake and regulation of its concentration in the synaptic cleft, the glycine transporter type 1 (GlyT1) may influence seizure activity and modulate hippocampal network function; however, knowledge in this area remains limited.

AIM(S): The aim of the study was to investigate the effect of SSR504734, a GlyT1 inhibitor, on the process of epileptogenesis and DNA methylation status in the amygdala kindling model in mice.

METHOD(S): CD-1 mice were divided into four groups: kindled and sham (non-kindled), receiving either SSR504734 at a dose of 30 mg/kg or saline throughout the kindling procedure. After completion of the kindling protocol, animals were sacrificed by decapitation, and the hippocampus and brainstem were collected for further analyses. Levels of DNA 5-mC and 5-hmC were determined using a colorimetric method.

RESULTS: SSR504734 did not inhibit the process of epileptogenesis in the amygdala kindling model. Changes in the levels of 5-mC and 5-hmC were observed in the hippocampus and brainstem in animals subjected to kindling and after administration of SSR504734, however, the differences were not statistically significant.

CONCLUSIONS: The presented results indicate that SSR504734 does not inhibit the process of epileptogenesis. However, the compound induced epigenetic changes in the brain by decreasing DNA methylation levels and increasing demethylation, which may suggest a potential influence on the process of epileptogenesis, although such functional effects were not observed.

FINANCIAL SUPPORT: Research project was funded by the National Science Centre, Poland (grant no. UMO-2021/41/NZ7/00328).

## S3P45. ANALYSIS OF THE TEMPORAL PROFILE OF CYSTATHIONINE BETA-SYNTHASE EXPRESSION IN RAT BRAINS IN RESPONSE TO SEIZURE ACTIVITY

Dominika Góral[1], Szymon Kantor[1,2], Zuzanna Setkowicz[1]

[1] *Laboratory of Experimental Neuropathology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Kraków, Poland*
[2] *Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Epilepsy is a complex neurological disorder characterised by an imbalance between excitatory and inhibitory neurotransmitters. Status epilepticus poses a serious health threat, leading to neuronal damage and long-term consequences. Given the significant number of patients with drug-resistant epilepsy, there is a need to explore new treatment strategies. Gaseous neurotransmitters, such as hydrogen sulfide, are gaining importance as potential therapeutic agents.

AIM(S): We examined the post-seizure temporal activation of $H_2S$ system. We propose that seizures increase the activity of the system.

METHOD(S): The study involved 75 male Wistar rats. Seizures were induced by administering methyl bromide scopolamine (1mg/kg,i.p.) followed by pilocarpine hydrochloride (250mg/kg,i.p.) after 30 minutes. Rats were observed for 6 hours, and seizure activity was assessed using a modified Racine scale. After designated survival times (6, 12, 24, 48, 96, and 192 hours), the rats were euthanised, and their hippocampi were isolated. Tissues were homogenised, and cystathionine β-synthase (CBS) expression was quantified via ELISA. Statistical analysis used the Kruskal-Wallis ANOVA and Dunn's *post hoc* test ($p<0.05$).

RESULTS: Our findings indicate that CBS expression levels peaked at 24 hours post-seizure induction, with significant elevations compared to untreated controls (5445.97[4638.56-6729.43]ng/mg *vs.* 280.75[34.51-359.53] ng/mg; $p<0.000001$). CBS expression was higher in severe seizure cases (6729.43 [6380.02-7404.21],n=5) compared to non-severe cases (4227.72[3535.36-4826.95], n=4) at 24 h.p.s.i., suggesting a correlation between seizure intensity and CBS activity.

CONCLUSIONS: These results highlight the potential neuroprotective role of $H_2S$ in the context of seizures, as increased CBS expression may contribute to an-

ti-apoptotic effects and reduction of oxidative stress in the brain. Further investigation into the mechanisms of action of $H_2S$ and CBS may open the way for new therapeutic strategies in drug-resistant epilepsy.

FINANCIAL SUPPORT: NCN Preludium Bis UMO-2021/43/O/NZ4/02208.

## S3P46. ALZHEIMER'S DISEASE BIOMARKERS IN UMBILICAL CORD BLOOD: LEVELS OF P-TAU217 AT BIRTH AND FUTURE AUTISM DIAGNOSIS IN THE MOBA COHORT

Fernando Gonzalez-Ortiz[1], Bjørn-Eivind Kirsebom[2], Julia K. Gundersen[2], Tormod Fladby[2]

*[1] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*
*[2] Department of Neurology, Akershus University Hospital, Lørenskog, Norway*

INTRODUCTION: Blood biomarkers such as p-tau217, GFAP, and NfL are typically interpreted in the context of brain injury or neurodegeneration, mainly Alzheimer's disease (AD). However, their biology at birth, and whether they relate to later neurodevelopmental outcomes remains unclear. In the present study, we evaluated umbilical cord blood biomarkers in the Norwegian Mother, Father and Child (MoBa) cohort.

AIM(S): To investigate the associations of AD-related biomarkers in umbilical cord blood and future neurodevelopment

METHOD(S): In this study (n=179), umbilical cord blood GFAP, plasma NfL, and plasma p-tau217 were analyzed using the Simoa HDX platform. We compared matched case–control sets for CP (cerebral palsy diagnosed at age 3 years) and autism (diagnosed at age 14 year). Additionally, we compared newborn biomarker levels against aged-controls and AD. Biomarker levels are presented as median [interquartile range]. Matched-set comparisons were performed using Wilcoxon test.

RESULTS: Consistent with prior reports, cord blood levels of p-tau217 were substantially higher in healthy newborns than in older adult controls and patients with AD (16.2 pg/ml [12.4 – 23.7] *vs.* 1.5 pg/ml [1.2 – 1.8] *vs.* 3.4 pg/ml [2.2 – 4.5] respectively, p<0.001). In the MoBa cohort, umbilical cord blood p-tau217 was significantly higher in newborns who were later diagnosed with autism compared with matched controls (22.0 pg/ml [12.2 – 25.] *vs.* 16.2 pg/ml [12.4 – 23.7], p=0.01, Fig. 1). No significant increases were observed in NfL (10.1 pg/ml [7.9 – 15.4] *vs.* 14.9 pg/ml [7.9 – 15.8], p=0.6, Fig. 2) or GFAP (642 pg/ml [285 – 926] *vs.* 691 pg/ml [402 – 980], p=0.2, Fig. 3). None of the markers evaluated showed a statistically significant increase in newborns later diagnosed with CP, although GFAP showed a non-significant increase compared with matched controls.

CONCLUSIONS: These findings suggest that, in newborns, neonatal p-tau217 may capture neurodevelopment-linked biology rather than classic neurodegenerative amyloid and tau pathology. Our results indicate that cord-blood p-tau217 could represent a window into early brain differences that precede later autism diagnosis. The neonatal p-tau217 differences may point to developmental pathways that could be leveraged for risk stratification and preventive/therapeutic strategies across the lifespan.

FINANCIAL SUPPORT: FG-O is funded by Hjärnfonden (#PD2025-0459), Alzheimerfonden (#AF-1032222) and Demensfonden (#DF-1031511).

## S3P47. FROM TANGLES TO BIOFLUIDS: CSF AND PLASMA PHOSPHO-TAU SERINE-262 AS A BIOMARKER OF TAU PATHOLOGY IN ALZHEIMER'S DISEASE

Fernando Gonzalez-Ortiz, Sophia Weiner, Johan Gobom, Gunnar Brinkmalm, Alicja Szadziewska, Henrik Zetterberg, Kaj Blennow

*Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*

INTRODUCTION: Central to tau pathology and tangle formation in Alzheimer's disease (AD) is the microtubule binding region (MTBR) of tau. MTBR fragments containing the first repeat (R1) harbour multiple phosphorylation sites that regulate tau-microtubule interactions and aggregation propensity. Among the three phospho-epitopes within the R1 domain (serine-258, serine-262, and threonine-263) serine-262 (p-tau262) stands out as critical epitope in both physiological brain development and pathological tangle formation.

AIM(S): To investigate the clinical utility of p-tau262 as a marker of tau pathology and tangle load in AD.

METHOD(S): We used mass spectrometry-based spatial proteomic profiling of laser-capture microdissected tangles from frozen hippocampal sections of AD patients. Tryptic peptides are analysed by LC-MS/MS, assessing peptide abundance changes in tangles versus

adjacent tissue (Fig. 1A). We further performed immunohistochemical staining of human brain to explore the colocalization of p-tau217 and p-tau262 across Braak stages. Finally, we developed a Simoa assay for p-tau262 based on the antibody used for the brain staining and two independent cohorts (n=542).

RESULTS: The untargeted proteomic analysis shows that certain tau posttranslational modifications are highly enriched in AT8-positive tangles compared to surrounding AT8-negative tissue, among these, phosphorylation at serine-262 was the main one present in the MTBR domain (Fig. 1B). Moreover, cortical immunostaining revealed extensive co-localization between p-tau217 and p-tau262, with p-tau262 having a more granular and nuclear pattern compared to p-tau217 (Fig. 2). In cohort-1, CSF p-tau262 showed a moderate correlation with CSF p-tau217 ($r=0.76$, $p<0.0001$) and significant increases in AD *vs.* controls. In the AD group CSF p-tau262 decreased over time in those with rapid clinical progression, compared to stable or slow cognitive decline (Fig. 3). In cohort-2, plasma p-tau262 concentrations were significantly higher in AD patients compared to non-AD individuals ($p<0.001$).

CONCLUSIONS: Our results underscore the importance of phosphorylation at serine-262 in tau tangle formation. We also present the first highly sensitive immunoassay for p-tau262 in CSF and plasma. The longitudinal CSF data suggest that declining p-tau262 may track rapid clinical deterioration, consistent with dynamic changes in tangle-associated tau pathology. Together, these findings position CSF and plasma p-tau262 as a biologically grounded, translatable biomarker of tau pathology with potential utility for staging, and disease monitoring in AD.

FINANCIAL SUPPORT: FG-O is funded by Hjärnfonden (#PD2025-0459), Alzheimerfonden (#AF-1032222) and Demensfonden (#DF-1031511).

## S3P48. MECHANISTIC INSIGHTS INTO GAMMA-SECRETASE CLEAVAGE OF AMYLOID-B PEPTIDES *VIA* STEERED MOLECULAR DYNAMICS SIMULATIONS

Emilia Wójcik[1,2], Urszula Orzeł[1,3], Paweł Pasznik[1], Sławomir Filipek[1]

[1] *Section of Inorganic and Analytical Chemistry, Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warszawa, Poland*

[2] *Inter-faculty Individual Studies in Mathematics and Natural Sciences, University of Warsaw, Warszawa, Poland*

[3] *PhD Programme in Biosciences, Department of Life Sciences, University of Coimbra, Coimbra, Portugal*

INTRODUCTION: Gamma-secretase (GS) is a multi-subunit intramembrane aspartyl protease that cleaves over 150 type I transmembrane proteins. Its catalytic core is formed by Presenilin. Among its substrates, the most extensively studied is the Amyloid precursor protein (APP). GS-mediated processing of APP generates amyloid-β peptides, predominantly Aβ40 and Aβ42. An increased Aβ42: Aβ40 ratio is a hallmark of Alzheimer's disease (AD), reflecting a shift toward the longer, more aggregation-prone Aβ42 species.

AIM(S): To develop an accessible computational model of GS proteolysis that enables mechanistic investigation of substrate cleavage and identification of crucial interactions stabilizing the substrate during catalysis.

METHOD(S): We performed Steered Molecular Dynamics (SMD) simulations using our GS-SMD server. Simulations were conducted in an implicit membrane and solvent environment to substantially reduce computational cost while preserving essential protein–substrate interactions.

RESULTS: Our simulations identified key interactions and specific residues within Presenilin that stabilize the substrate during cleavage. These interaction networks appear critical for maintaining substrate positioning within the catalytic site.

CONCLUSIONS: We established a computationally efficient model that provides mechanistically meaningful insight into GS-mediated proteolysis. Importantly, several identified stabilizing residues in presenilin correspond to sites known to harbor pathogenic mutations in familial AD, supporting the biological relevance of our approach.

FINANCIAL SUPPORT: This research was conducted without external financial support.

## S3P49. CARGO-SPECIFIC REGULATION OF MICROGLIAL AB PHAGOCYTOSIS BY LIPID-RESPONSIVE TRANSCRIPTION FACTOR SREBF2

Izabela Lepiarz-Raba[1], Taufik Hidayat[2], Weronika Tomaszewska[2], Ali Jawaid[2], Sandra Binias[3], Bartłmiej Gielniewski[3], Jacek Miłek[4], Magdalena Dziembowska[4]

[1] *Translational Research in Exposures and Neuropsychiatric Disorders (TREND), Nencki Institute of Experimental Biology Polish Academy of Sciences, Warsaw, Poland*
[2] *Translational Neuropsychiatry Research Group, Łukasiewicz-PORT, Wrocław, Poland*
[3] *Laboratory of Sequencing, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland*
[4] *Department of Biology, University of Warsaw, Warsaw, Poland*

INTRODUCTION: Alzheimer's disease (AD), the leading cause of dementia, is marked by abnormal accumulation of amyloid-β (Aβ) in the brain. Microglia, the brain's resident immune cells, are responsible for Aβ clearance, and their phagocytic function is strongly influenced by metabolic and environmental states. Understanding how metabolic manipulation affects microglial Aβ handling may provide a route to selectively enhance pathological Aβ clearance while preserving healthy neuronal structures.

AIM(S): To investigate how metabolic perturbations, specifically lipid starvation and serum starvation modulate microglial Aβ uptake and degradation, and to identify molecular pathways regulating cargo-specific Aβ phagocytosis, with a focus on the role of SREBF2.

METHOD(S): Human HMC3 microglia were subjected to lipid starvation (medium delipidation) and serum starvation. Aβ uptake and degradation were analyzed under both conditions.In addition, transcriptomic profiling was performed using RNA sequencing to identify starvation-induced pathway changes. Lastly, functional validation included siRNA-mediated knockdown of SREBF2 to assess its role in lipid-starvation–enhanced Aβ phagocytosis and cargo specificity.

RESULTS: Both lipid and serum starvation induced major transcriptomic changes, particularly in cholesterol biosynthesis, SREBF signaling, and steroid metabolism pathways, and although Aβ uptake increased under both conditions, efficient degradation occurred only during lipid starvation, whereas serum starvation impaired degradation and led to intracellular Aβ accumulation; furthermore, knockdown of SREBF2 abolished the lipid starvation–induced enhancement of Aβ phagocytosis without affecting neurosynaptosome uptake, demonstrating cargo-specific regulation.

CONCLUSIONS: SREBF2-dependent cholesterol metabolic signaling is a key regulator of Aβ uptake and degradation. This pathway represents a promising and selective therapeutic target for enhancing microglial Aβ clearance in AD.

FINANCIAL SUPPORT: EU Joint Programme – Neurodegenerative Disease Research (JPND) [TREMENDOS; UMO-2022/04/Y/NZ5/00122].

## S3P50. EFFECT OF TRIS(2,3-DIBROMOPROPYL)ISOCYANURATE ON STEROIDOGENESIS IN MOUSE HIPPOCAMPAL NEURONAL CELLS

Anna Tabęcka-Łonczyńska, Dominika Szlachcikowska

*Department of Biotechnology and Cell Biology, Medical College, University of Information Technology and Management in Rzeszów, Rzeszów, Poland*

INTRODUCTION: Tris(2,3-dibromopropyl) isocyanurate (TBC; TAZTO) is a persistent, lipophilic brominated flame retardant that is resistant to biodegradation and undergoes bioaccumulation in the environment and in the tissues of organisms. It can also accumulate in the central nervous system, affecting cellular function. To date, TBC has been shown to disrupt the synthesis and action of steroid hormones, receptor signaling pathways, and metabolic processes. Progesterone, testosterone, and estradiol play a key neuroprotective role in the nervous system by regulating neuronal survival, the response to oxidative stress, and cellular metabolic homeostasis.

AIM(S): Therefore, the aim of this study was to assess the neurotoxic effects of TBC in murine neuronal cells (HT-22).

METHOD(S): The effect of TBC on the synthesis of progesterone, testosterone, and estradiol was analyzed using ELISA, both in the cells and in the culture medium. In addition, the expression of estrogen receptors (ERα and GPER) was evaluated by Western blot analysis.

RESULTS: The obtained results indicate that TBC may disrupt steroid hormone synthesis.

CONCLUSIONS: suggesting impairment of neuroprotective mechanisms in the murine HT-22 cellular model.

FINANCIAL SUPPORT: This work was supported by statutory funds from the University of Information Technology and Management in Rzeszow, Poland (DS 503-07-01-59).

## S3P51. PRELIMINARY EVALUATION OF NEUROTOXIC EFFECTS OF TRIS(2,3-DIBROMOPROPYL) ISOCYANURATE (TBC) IN MOUSE HIPPOCAMPAL HT-22 CELLS

Dominika Szlachcikowska, Anna Tabęcka-Łonczyńska

*Department of Biotechnology and Cell Biology, Medical College, University of Information Technology and Management in Rzeszów, Rzeszów, Poland*

INTRODUCTION: Tris(2,3-dibromopropyl)isocyanurate (TBC) is a novel brominated flame retardant widely used in industry and increasingly detected in environmental samples and living organisms. Its lipophilic character suggests the ability to penetrate the blood–brain barrier, raising concerns about potential neurotoxic effects. However, data regarding the impact of TBC on neuronal cells and the underlying mechanisms remain limited.

AIM(S): The aim of this study was to evaluate the influence of TBC on mouse hippocampal HT-22 cells as an in vitro model and to obtain preliminary insight into cellular pathways involved in the response to this compound.

METHOD(S): A broad methodological approach was applied, including fluorescence and immunofluorescence staining, plate-based metabolic assays, flow cytometry, and Western blot analysis. The assessment focused on mitochondrial function, cellular metabolic activity, stress-related signaling, and processes associated with cell death and autophagy.

RESULTS: Preliminary results indicate that exposure to TBC leads to disturbances in mitochondrial integrity and a decline in metabolic activity of HT-22 cells, accompanied by activation of cellular stress response. Additional observations suggest alterations in lysosomal compartment and modulation of proteins linked with autophagic and apoptotic pathways.

CONCLUSIONS: These findings point to a potentially negative impact of TBC on hippocampal neurons and highlight the need for further studies to clarify its mechanism of action and relevance for neurodegenerative processes.

FINANCIAL SUPPORT: The study was supported by statutory funds from the University of Information Technology and Management in Rzeszow, Poland (DS 503-07-01-59).

## S3P52. COPPER CHELATION *VIA* MITOCHONDRIA-TARGETED NANOSTRUCTURED LIPID CARRIERS TO MITIGATE OXIDATIVE STRESS IN NEURODEGENERATIVE DISORDERS

Akshada Mhaske[1], Kamlinder K singh[2,3], Rahul Shukla[1]

[1] *Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research – Raebareli, Lucknow, India*

[2] *School of Pharmacy and Biomedical Sciences, University of Central Lancashire, Preston, United Kingdom*

[3] *Biomedical Evidence-based Transdisciplinary (BEST) Health Research Institute, University of Central Lancashire, Preston, United Kingdom*

INTRODUCTION: Copper is an essential trace element critical for ATP production through the mitochondrial electron transport chain. Dysregulation and accumulation of copper, as observed in Wilson's disease and several neurodegenerative disorders, disrupt cellular homeostasis, induce oxidative stress, and impair mitochondrial function. Strategies that selectively target mitochondria are therefore crucial to restore neuronal health.

AIM(S): To develop and evaluate a mitochondria-targeted nanostructured lipid carrier (NLC) capable of co-delivering a copper chelator and antioxidant to mitigate copper-induced mitochondrial dysfunction in neuronal cells.

METHOD(S): A mitochondria-targeted NLC was engineered with a mitochondrial-targeting ligand to enhance delivery to neuronal mitochondria. The NLC co-delivers D-penicillamine and kaempferol for synergistic copper chelation and antioxidant activity. Surface modification facilitated mitochondrial localization, and the lipid composition supported blood-brain barrier penetration in cellular models. Protective effects were assessed in SH-SY5Y and HepG2 cells. Copper chelation and cytoprotection were evaluated in ATP7B knockdown models. Mechanistic studies included RT-PCR for mitochondrial and oxidative stress markers, site-directed uptake assays, ATP quantification, and mitochondrial functional analyses.

RESULTS: The NLC effectively redirected excess copper and attenuated cytotoxicity in ATP7B knockdown cells. Mitochondrial protection was confirmed by restored ATP levels and functional assays, with reduced oxidative stress mediated via activation of the SIRT1/PGC-1α pathway.

CONCLUSIONS: This dual-function NLC enables prolonged circulation, controlled release, and efficient intracellular delivery. By combining copper chelation with antioxidant therapy, it represents a promising strategy to mitigate mitochondrial dysfunction and enhance neuronal survival in Wilson's disease and related neurodegenerative disorders.

FINANCIAL SUPPORT: Commonwealth scholarship (United Kingdom).

## S3P53. CANNABINOID RESCUE OF MEMORY DEFICITS THROUGH ASTROGLIAL LIPID METABOLISM IN ALZHEIMER'S DISEASE

Carla Ramón-Duaso[1], Júlia S. Pinho[2], Xoel Mato-Blanco[3], Pau Berenguer-Molins[3], Júlia Perera-Bel[3], Arnau Busquets-Garcia[1]

[1] *Cell-type mechanisms in normal and pathological behavior, Hospital del Mar Research Institute, Barcelona, Spain*

[2] *Gulbenkian Institute for Molecular Medicine, Oeiras, Portugal*

[3] *MARData-BU, Hospital del Mar Research Institute, Barcelona, Spain*

INTRODUCTION: Astrocytes contribute to Alzheimer's disease (AD) through metabolic and functional alterations, yet the link between astroglial dysfunction, memory deficits, and cannabinoid signaling remains unclear.

AIM(S): To investigate sex-, genotype-, and cannabinoid-dependent effects on memory and astrocyte biology in the APP/PS1 mouse model, focusing on astroglial lipid metabolism.

METHOD(S): Male and female wild-type and APP/PS1 mice received chronic cannabinoid treatment during the presymptomatic stage and were assessed longitudinally using the Novel Object Recognition Task and contextual fear conditioning. Astrocytic activity in dorsal CA1 was selectively modulated during memory consolidation. In vivo fiber photometry monitored hippocampal astrocytic calcium dynamics during behavior. Single-cell RNA sequencing characterized astrocyte-specific transcriptional changes related to lipid metabolism.

RESULTS: Male APP/PS1 mice showed recognition memory deficits that were prevented by cannabinoid treatment, while female mice exhibited fear memory impairments that were not rescued. Modulation of dorsal CA1 astrocytes restored recognition memory. Fiber photometry revealed sex-, genotype-, and cannabinoid-dependent alterations in astrocytic calcium signaling. Single-cell RNA sequencing identified disrupted astroglial lipid metabolism in male APP/PS1 mice that was normalized by cannabinoid treatment.

CONCLUSIONS: Cannabinoid treatment preserves memory in association with restored astroglial lipid metabolism and calcium signaling, highlighting astrocytes as key mediators of cognitive deficits and therapeutic targets in AD.

FINANCIAL SUPPORT: Project RTI2018-093667-A-100.

## S3P54. NEUROIMMUNOMODULATORY EFFECTS OF THE OXYSTEROL CF3-7A,25OHC IN MICE MODELS OF MULTIPLE SCLEROSIS

Klaudia Konieczna-Wolska[1], Fiona Caratis[1], Mikołaj Opiełka[1], Karol Biernacki[2], Aleksandra Rutkowska[1]

[1] *Department of Anatomy and Neurobiology, Medical University of Gdańsk, Gdańsk, Poland*

[2] *Department of Organic Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland*

INTRODUCTION: The Epstein-Barr virus-induced gene 2 (EBI2/GPR183) is a G protein-coupled receptor primarily expressed in the immune system, but also functional in astrocytes, microglia, and oligodendrocytes. Its most potent endogenous agonist, the oxysterol CF3-7α,25-dihydroxycholesterol, directs immune cell migration and has been implicated in the pathophysiology of multiple sclerosis (MS). Previous studies demonstrated that EBI2 deficiency leads to impaired remyelination and increased demyelination. However, the short half-life of natural oxysterols limits their therapeutic application. Therefore, we investigated a biostable synthetic analogue,CF3-7α,25-OHC, to evaluate its neuroimmunomodulatory and regenerative potential in MS models.

AIM(S): The study aims to evaluate the neuroimmunomodulatory and regenerative potential of a metabolically stable EBI2 receptor agonist, CF3-7α,25OHC. The research focuses on determining whether this compound can accelerate remyelination and modulate the autoimmune response in two distinct mice models of multiple sclerosis (MS).

METHOD(S): – Cuprizone Model: Male C57BL/6 mice were fed a 0.2% cuprizone diet for 9 weeks to induce toxic demyelination, followed by 2 weeks of CF3-7α,25OHC treatment. – EAE Model: Experimental Autoimmune Encephalomyelitis (EAE) is induced to mimic the autoimmune component of MS. Following pilot studies in SJL/J mice, the current project involves EBI2 knock-out (KO) mice. – Clinical Assessment: Disease progression in EAE is monitored daily using a clinical scoring scale (0 to 4.5), ranging from a limp tail to complete paralysis. – Analytical Techniques: Evaluation includes Luxol Fast Blue (LFB) staining for myelin density, qPCR for gene expression and blood count analysis.

RESULTS: – Accelerated Remyelination: In the cuprizone model, CF3-7α,25OHC significantly accelerated remyelination in the corpus callosum compared to vehicle-treated controls. – Immune Modulation: Treatment led to a pronounced reduction in peripheral white

blood cell counts, with lymphocytes decreasing by 51% and monocytes by 66%. – Molecular Changes: The analogue upregulated Ebi2 expression in the brain and increased the synthesis of 15 lipid classes in the corpus callosum. – EAE Expectations: Preliminary data from EBI2/KO models (to be conducted in April) are expected to clarify the receptor's role in clinical onset and peak disability during autoimmune neuroinflammation.

CONCLUSIONS: The findings demonstrate that CF3-7α,25OHC acts as a dual-action agent by promoting myelin repair and exerting potent systemic immunomodulatory effects. Bridging the results from toxic and autoimmune models validates the EBI2/oxysterol axis as a promising therapeutic target for the treatment of both neurodegenerative and inflammatory phases of multiple sclerosis.

FINANCIAL SUPPORT: This project received funding from the National Science Centre, Poland, grant registration number: 2022/47/D/NZ3/02613.

## S3P55. EFFECTS OF HUMAN SOD1 GENE EXPRESSION IN ALL NEURONS ON THE SURVIVAL AND FITNESS OF DROSOPHILA MELANOGASTER

Khuslenzaya Hunter, Elżbieta Pyza

*Department of Cell Biology and Imaging, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Superoxide dismutase 1 (SOD1) binds Cu/Zn and is located in the cytoplasm. It is one of three enzymes of a family of SODs which catalyse the dismutation reaction of superoxide to hydrogen peroxide. Mutations of the SOD1 in humans lead to amyotrophic lateral sclerosis (ALS), a neurodegenerative disorder that is characterized by the progressive injury of motor neurons. Under pathological conditions, hSOD1 variants go through changes in conformation, leading to protein misfolding and the formation of neurotoxic aggregates. By expressing the hSOD1 gene within the Drosophila melanogaster nervous system, we can observe how the molecular changes cause the declines in lifespan and fitness.

AIM(S): The aim of the study was to investigate the effects of pan-neuronal expression of human superoxide dismutase 1 gene on the survival and fitness of Drosophila melanogaster.

METHOD(S): Pan-neuronal expression of hSOD1 was achieved using the UAS-Gal4 system by crossing elav-Gal4 (driver) and UAS-hSOD1 (responder) lines. Controls were established by crossing both lines to a w1118 (white) background. The transgenic flies were kept on a standard cornmeal medium. Survival was monitored every day. Flies were transferred to a fresh medium twice per week. Fitness was evaluated by the negative geotaxis assays on 7, 15, 30, and 50 day old flies. Flies were tapped to the bottom of empty vials under dim red light. The number of individuals crossing the threshold (12,5 cm) within 15s was recorded. The test was repeated 3 times per group/sex.

RESULTS: The negative geotaxis assay showed a decrease in the fitness of both sexes of the transgenic flies with age.

CONCLUSIONS: Expression of the human Cu/Zn superoxide dismutase gene in Drosophila melanogaster neurons affects their survival and fitness.

FINANCIAL SUPPORT: NCN project nr 2020/39/B/NZ7/03366.

## S3P56. CHANGES IN THE SYNAPTIC PLASTICITY AND EXCITABILITY OF THE SOMATOSENSORY CORTEX IN A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS AFTER SWIM TRAINING

Anbarieh Saadat[1], Małgorzata Jasińska[2], Bartosz Cedro[3], Alicja Piekarska[3], Damian Flis[3], Wiesław Ziółkowski[4], Elżbieta Pyza[1]

[1] *Department of Cell Biology and Imaging, Jagiellonian University, Kraków, Poland*

[2] *Department of Histology, Jagiellonian University Medical College, Kraków, Poland*

[3] *Department of Pharmaceutical Pathophysiology, Medical University of Gdańsk, Gdańsk, Poland*

[4] *Department of Rehabilitation Medicine, Medical University of Gdańsk, Gdańsk, Poland*

INTRODUCTION: Amyotrophic Lateral Sclerosis (ALS) is a progressive and incurable neurodegenerative disease, and treatments such as physical activity are focused on managing and reducing the symptoms of the disease. The hyperexcitability in the somatosensory cortex is observed during the presymptomatic stage of ALS.

AIM(S): This study aimed to understand how excitation develops throughout the disease and whether swim training has any effect on hyperexcitation.

METHOD(S): Here, we analysed the density of excitatory and inhibitory synapses, their ratio (E/I), and dendritic spine morphology in the B2 barrel of the somatosensory cortex in female SOD1G93A mice using transmission electron microscopy. ALS mice were divided and analysed before the onset of the disease, at the onset, and at the terminal stage with, and without swim training. Swim training was applied after the first onset of symptoms.

RESULTS: The obtained results showed an increase in the density of excitatory synapses in the presymptomatic ALS mice, consistent with early cortical hyperexcitability. At disease onset, we detected an increase in inhibitory synapses, suggesting a compensatory mechanism that may act to stabilise network activity. Dendritic spine morphology also changed across stages, reflecting structural remodelling accompanying the evolving synaptic imbalance. Swim training reduced excitatory synapse density and lowered the E/I ratio in symptomatic ALS mice, indicating a shift toward decreased cortical excitation.

CONCLUSIONS: Our data confirm cortical hyperexcitability and changes in the synaptic balance in female ALS mice and suggest the potential benefit of swim training as an activity-based strategy for managing cortical dysfunction in ALS.

FINANCIAL SUPPORT: NCN OPUS 20 nr UMO-2020/39/B/NZ7/03366 to WZ and EP.

## S3P57. LONG-TERM CULTURE OF PATIENT-DERIVED STRIATAL ORGANOIDS AS AN *IN VITRO* MODEL OF HUNTINGTON'S DISEASE

Wioletta Lech[1], Magdalena Czeredys[1], Erkan Metin[1], Zuzanna Kuczyńska[1], Paweł Leszczyński[1], Aleksandra Duchnowska[2], Marzena Zychowicz[1], Leonora Bużańska[1]

[1] *Department of Stem Cell Bioengineering, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland*

[2] *Laboratory of Molecular Basis of Neurodegeneration, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland*

INTRODUCTION: Huntington's disease (HD) is a genetic condition caused by an expansion of the CAG trinucleotide repeat in the HTT gene, which encodes the huntingtin protein. An excessive number of CAG repeats leads to cellular alterations, including mitochondrial dysfunction and reduced ATP levels, ultimately resulting in progressive neuronal degeneration and cell death in the striatum and cerebral cortex.

AIM(S): In vitro brain organoid cultures are a valuable platform for modelling the progression and pathogenesis of neurodegenerative diseases. We were analysed control and HD patient-derived striatal organoids to verify differences in their morphology, organisation and viability.

METHOD(S): iPSCs were seeded onto AggreWell plates to generate spheroids. Neural induction was initiated by administering Dual SMAD inhibitors. Striatal differentiation was induced by the addition of Activin A, IWP-2 (a Wnt signalling inhibitor) and SR11237 (a retinoid X receptor agonist). Further maturation was promoted through the following supplementation: BDNF, NT3, ascorbic acid, cAMP and docosahexaenoic acid. Organoid viability was assessed during long-term in vitro culture using an Alamar Blue (AB) assay. 100-day-old organoids were fixed and sectioned into 20 µm-thick slices using a cryostat. To confirm striatal identity, expression of FOXP1, DARPP-32, GABA and GAD65+67 proteins was analysed. Furthermore, the expression levels of HUNTINGTIN, NESTIN and βTUBULINIII were evaluated.

RESULTS: At day 100, the mean organoid diameter ranged from 1.2 to 1.4 mm. The results of the AB assay demonstrated high viability of the organoids. All organoids expressed FOXP1, DARPP-32, GABA and GAD65+67, confirming a striatal phenotype. HUNTINGTIN protein, as well as neural markers NESTIN and βTUBULINIII were detected.

CONCLUSIONS: The utilised protocol enables reproducible and efficient generation of striatal organoids from control and HD samples. These organoids provide an in vitro system for modelling neurodegeneration in Huntington's disease.

FINANCIAL SUPPORT: The work was supported by the Medical Research Agency grant no. KPOD.07.07-IW.07-0105/25.

## S3P58. INDIVIDUAL DIFFERENCES IN PUPILLARY DYNAMICS UNDER INCREASING VISUAL STIMULATION

Beata Pacula

*Institute of Psychology, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Pupil size adaptation is a well-established marker of autonomic nervous system activity that offers valuable insights into physiological correlates of both sensory and emotional processing in a nonobtrusive way.

AIM(S): The present study investigates whether basic visual stimuli with increasing luminance can induce different autonomic responses in individuals with varying levels of sensory processing sensitivity.

METHOD(S): We used light ramps—grayscale full-screen images created in which luminance increased gradually in fixed increments, creating a transition from low to high salience. These stimuli were designed to isolate luminance-driven responses while minimizing cognitive load and distraction. We have tested 105 participants (55 women). To display the stimuli, we used an HTC Vive Pro headset with a dedicated 200Hz Pupil Labs eye-tracking system to record the pupil size. Sensory Processing Sensitivity was measured using the Highly Sensitive Person Scale for research purposes.

RESULTS: Pupil responses were significantly influenced by stimulus intensity progression and baseline pupil size, showing a pronounced nonlinear adaptation pattern across increasing luminance. Importantly, Sensory Processing Sensitivity moderated this relationship: individuals with high sensory processing sensitivity scores have significantly weaker pupillary constriction in response to high-luminance displays compared to those with lower sensitivity.

CONCLUSIONS: Attenuated adaptive response suggests a modulation of parasympathetic activity and potentially reduced autonomic adaptability under sensory load. The other possible explanation lies in the elevated activity of the sympathetic nervous system, that could stop the pupil from full constriction. The findings highlight the potential of pupillometry as a non-invasive tool for assessing individual differences in sensory processing and contribute to a growing body of research on the physiological correlates of neurodiversity.

FINANCIAL SUPPORT: This work was supported by the Polish National Science Centre (grant number 2021/41/N/HS6/04490).

## S3P59. EMOTION REGULATION IN HEALTHY YOUNG MALES IS MODERATED BY CORTISOL AND TESTOSTERONE-TO-CORTISOL RATIO

Erik Ilkevič[1], Ingrida Zelionkaitė[1], Eglė Mazgelytė[2], Violeta Cimalanskaitė-Kazlauskienė[3], Ramunė Grikšienė[1]

[1] *Department of Neurobiology and Biophysics, Institute of Biosciences, Vilnius University, Vilnius, Lithuania*

[2] *Faculty of Medicine, Institute of Biomedical Sciences, Vilnius University, Vilnius, Lithuania*

[3] *Faculty of Philosophy, Institute of Psychology, Vilnius University, Vilnius, Lithuania*

INTRODUCTION: Emotion regulation refers to the processes through which individuals modify their emotional states using various strategies. One widely studied strategy is cognitive reappraisal, involving reinterpretation of emotional stimuli to alter emotional responses. Evidence suggests that cortisol is related to more effective cognitive reappraisal, whereas the links with testosterone remain unclear. Because cortisol and testosterone mutually inhibit each other's activity, understanding how they interact would provide a broader understanding of how they may be related to emotional behavior.

AIM(S): The aim of the study was to evaluate whether the interplay between testosterone and cortisol is associated with cognitive reappraisal performance.

METHOD(S): 140 males completed an emotion regulation task, where they either passively viewed or reappraised emotional visual stimuli. After each stimulus, participants reported their emotional negativity. The reduction in self-reported negativity from the viewing to the reappraisal condition served as an index of the effectiveness of cognitive reappraisal. Participants also completed a questionnaire assessing habitual use of cognitive reappraisal and provided saliva samples to assess basal (i.e., before the task) cortisol and testosterone concentrations.

RESULTS: Higher habitual use of cognitive reappraisal was associated with greater reductions in self-reported negativity when basal cortisol levels were high and the testosterone-to-cortisol ratio was low (indicating lower testosterone and higher cortisol levels). Furthermore, the testosterone-to-cortisol ratio showed a stronger relationship with emotion regulation performance than cortisol alone.

CONCLUSIONS: Higher basal cortisol levels were associated with more effective cognitive reappraisal,

with testosterone potentially attenuating this relationship. Study results highlight the importance of considering an individual's hormonal profile and hormonal interactions when examining vulnerabilities related to emotion regulation.

FINANCIAL SUPPORT: This work was supported by Vilnius University Research Promotion Fund (project code: MSF-JM-12/2024).

## S3P60. TESTING THE POTENTIAL OF READING TIME FROM AN ANALOGUE CLOCK AS A MODEL OF VISUAL EXPERTISE

Magdalena Miśkowiec[1], Marcin Furtak[2], Anna Rachowicz[1], Marek A. Pedziwiatr[1]

[1] *Centre for Brain Research, Jagiellonian University, Kraków, Poland*

[2] *Institute of Cognitive Science, Osnabrück University, Osnabrück, Germany*

INTRODUCTION: Studying visual expertise — that is, the proficiency in various vision-related domains — poses significant methodological challenges. In some domains, the population of experts is small (e.g. in chess). Other domains, such as face perception, are characterised by a limited variability in the level of expertise found in non-clinical populations. Laboratory studies involving training participants to become experts in novel domains are, in turn, time-consuming and offer limited ecological validity.

AIM(S): Here, we explore if reading time from an analogue clock has the potential to be a model for studying visual expertise that does not require overcoming the above-mentioned challenges and possesses other desired properties: the ability to perform this task likely varies naturally across the general population, which should allow for conducting ecologically valid studies in large, varied samples.

METHOD(S): To test whether clock reading is indeed a good model for visual expertise research, we are currently conducting an eye-tracking experiment including two tasks: a time-telling task, in which participants report the time displayed on a clock, and a parity task, in which they report whether a cued number on a clock face is odd or even. Importantly, this number can be either the number expected at the given position or not.

RESULTS: We hypothesize that experts (individuals with a higher performance in the time telling-task), as compared to non-experts, will exhibit 1) more pronounced oculomotor hallmarks of visual expertise (e.g. shorter fixation durations and more selective attention allocation) and 2) impaired performance in the parity task resulting from greater difficulty in processing the numbers they do not expect. The data collection is still ongoing.

CONCLUSIONS: Nevertheless, our study has the potential to test if reading time from an analogue clock constitutes a useful model of visual expertise, facilitating further investigation of this phenomenon.

FINANCIAL SUPPORT: This research was funded by a grant from the National Science Centre, Poland (2024/55/D/HS6/02157) awarded to the author M.P.

## S3P61. DEVELOPMENT OF A TOOL FOR ASSESSING ANALOGUE-CLOCK READING SKILLS

Anna Rachowicz[1], Magdalena Miśkowiec[1], Antonia Franaszek-Traczewska[2], Marek A. Pedziwiatr[1]

[1] *Centre for Brain Research, Jagiellonian University, Kraków, Poland*

[2] *Neurocognitive and Experimental Psychology Lab, Freie Universität Berlin, Berlin, Germany*

INTRODUCTION: The ability to read time from an analogue clock is acquired in childhood with the support of formal education and is considered so ubiquitous that analogue clocks feature prominently in public spaces, while the impaired ability to draw (and read) them is treated as an indicator of possible mental decline. The increased exposure to digital clocks that stems from the recent technological changes, such as the proliferation of smartphones, leads to the question of whether the assumption about the ubiquity of the ability to read time from an analogue clock in the general population still holds. Few studies have explored this topic, and currently, there is no standard testing instrument for assessing human performance in clock reading.

AIM(S): Our ongoing project aims to fill this gap and create such a tool. Along the way, we intend to leverage the inherent resemblance of clock reading to classic cognitive psychology tasks to answer basic questions about visual processing by, for example, analysing the patterns of errors in clock reading in relation to the characteristics of times shown on the clock faces (e.g. display of whole hours *vs.* hours and minutes).

METHOD(S): Our first step involves a large-scale online experiment that assesses the difficulty of reading 144 different times separated by five-minute intervals.

On each trial of this experiment, participants read the time from a numeral-free clock presented for two seconds and judge how difficult it was to read.

RESULTS: Initial results from 120 participants show higher accuracy for reading minutes, which, interestingly, does not reflect the order of development of clock reading skills in children, which is characterized by the earlier acquisition of knowledge about the clock's hour hand.

CONCLUSIONS: Our data will contribute to the creation of a new tool for quantifying the ability to read time from an analogue clock and provide insights into the perceptual processes involved in this complex visual task.

FINANCIAL SUPPORT: This research was funded by a grant from the National Science Centre, Poland (2024/55/D/HS6/02157) awarded to the author M.P.

## S3P62. TEMPORAL DYNAMICS OF VISUAL AWARENESS: HOW PRESENTATION TIME MODULATES SPATIAL FREQUENCY PERCEPTION IN NATURALISTIC PERCEPTUAL RIVALRY

Zofia Kołaczkowska[1,2], Marek A. Pedziwiatr[1], Michał Bola[1]

[1] *Centre for Brain Research, Jagiellonian University, Kraków, Poland*

[2] *Faculty of Biology, Jagiellonian University, Kraków, Poland*

INTRODUCTION: Scene perception relies on parallel subcortical pathways having different temporal dynamics and processing different kinds of information. Specifically, low spatial frequencies (LSF), conveying the global 'gist' of a scene, are handled by the rapid magnocellular pathway, while high spatial frequencies (HSF), carrying scene details, pass through the slower parvocellular pathway. Here, we investigated how these pathways interact. To this end, we harnessed perceptual rivalry – a state occurring when the brain receives conflicting information and resulting in perceiving competing interpretations of the same sensory input alternately.

AIM(S): We aim to induce perceptual rivalry between naturalistic scenes and use it to determine how stimulus presentation time modulates the processing of HSF and LSF.

METHOD(S): We developed 'hybrid' stimuli by superimposing scenes from two categories. In each hybrid, one category contained only LSF components, while the other only HSF. In the first experiment, involving 15 participants and a presentation time of 133 ms, we used a staircase procedure adjusting intensities of both categories for each observer so that the hybrids elicited perceptual rivalry (defined as a ~50% chance of perceiving each category). In a follow-up experiment, we tested whether shorter presentation times shift the balance between LSF and HSF components.

RESULTS: The first experiment demonstrated that the staircase procedure successfully induces perceptual rivalry. Ongoing analyses of the data from the second experiment suggest that shorter times significantly alter the intensities required to achieve the 50/50 perceptual equilibrium between the LSF and HSF.

CONCLUSIONS: Analysing the requirements for rivalry across temporal scales reveals how the brain balances competing representations under varying time constraints. Consequently, it offers insights into the functional properties of visual awareness and the temporal profiles of LSF and HSF processing.

FINANCIAL SUPPORT: This study was funded by the Sonata Bis grant (2022/46/E/HS6/00150) awarded to the author M. B. by the National Science Centre, Poland.

## S3P63. NORMATIVE PATTERNS OF CHANGE IN VISUOSPATIAL FUNCTIONS IN HEALTHY AGING

Weronika Sobolewska, Ewa Malinowska

*Faculty of Psychology, University of Warsaw, Warszawa, Poland*

INTRODUCTION: Visuospatial abilities encompass the perception, representation, and manipulation of spatial relationships and constitute a core domain of cognitive functioning. Although cognitive aging is well documented, the pattern of age-related changes across specific visuospatial components in healthy adults remains insufficiently defined.

AIM(S): This study aimed to provide a quantitative characterization of selected visuospatial processes and to examine their associations with age.

METHOD(S): Fifty-eight neurologically and psychiatrically healthy adults completed measures of visual search (Test of Attention and Perceptiveness; Color Trails Test 1 and 2), mental rotation (Mental Rotation Test – Version A), spatial perception (Line Orientation Recognition task), and spatial visualization (Block Design, WAIS-IV). Accuracy and completion time indices were analyzed using Spearman's rho correlations.

RESULTS: Age was moderately and negatively associated with performance accuracy in mental rotation

(rho=−0.35, p<.05) and spatial perception (rho=−0.31, p<.05), indicating increased error rates with advancing age. No significant association was observed between age and completion time in visual search tasks.

CONCLUSIONS: These findings suggest that normative aging is characterized by greater vulnerability of processes requiring manipulation and discrimination of spatial relationships, whereas processing speed in visual search appears relatively preserved. The pattern supports partial selectivity of age-related changes rather than uniform visuospatial decline. The results provide a reference framework for differentiating normative aging from pathological visuospatial impairment in clinical assessment.

FINANCIAL SUPPORT: Fundusz Wspierania Inicjatyw Naukowych Studentów Wydziału Psychologii.

## S3P64. USING AI TO GENERATE AFFECTIVE IMAGES: METHODOLOGY AND INITIAL LIBRARY

Rafał Jończyk[1,2], LAI-GAI team[3]

[1] *Faculty of English, Adam Mickiewicz University, Poznań, Poland*
[2] *Cognitive Neuroscience Center, Adam Mickiewicz University, Poznań, Poland*
[3] *LAI-GAI team*

INTRODUCTION: Image-based affect induction research is constrained by weak to moderate emotional elicitation effects, limited stimulus diversity, and minimal cultural tailoring. Advances in generative AI offer new opportunities to produce scalable, customizable, and context-aware affective stimuli.

AIM(S): We developed a human-in-the-loop pipeline for generating context-aware affect induction images and established and validated the initial Library of AI-Generated Affective Images (LAI-GAI), with stimuli tailored to cultural, sex, and age contexts.

METHOD(S): Using generative AI guided by established emotion taxonomies and existing affective datasets, we produced 847 images and corresponding descriptions spanning 12 discrete emotions. Images were iteratively refined in collaboration with local cultural experts. Validation was conducted across six studies (total N=2,470; participants from 58 countries). Participants rated five image categories: (1) images from existing affective databases, (2) AI-generated images without cultural adjustment, (3) culturally adjusted AI-generated images, (4) sex-adjusted variants (male, female), and (5) age-adjusted variants (childhood, adulthood, older age).

RESULTS: AI-generated images were comparable to established database images in eliciting affective responses. Culturally adjusted images showed greater effectiveness in targeting intended emotions than unadjusted AI images. Sex- and age-adjusted variants produced affective responses comparable to their base images, indicating controllability without diminished emotional impact. We also estimated the smallest subjectively experienced difference for affect induction research (Cohen's d=0.05–0.29).

CONCLUSIONS: The LAI-GAI demonstrates that high-quality, scalable, and context-sensitive affect induction stimuli can be generated cost-effectively using a human-in-the-loop AI pipeline. This approach overcomes longstanding limitations in affective research and establishes a foundation for future AI-driven methodologies in affective science.

FINANCIAL SUPPORT: National Science Centre in Poland (UMO-2020/39/B/HS6/00685; 2023/49/B/HS5/02139) and Excellence Initiative—Research University (ID-UB) program at Adam Mickiewicz University, Poznan (140/04/POB5/0001, 151/12/POB5/0005, 174/12/POB5/0001, 177/02/UAM/0012, 181/13/SNS/0003, 198/12/POB5/0007) supported preparing this article with a research grant. The funders had no role in study design, data collection, analysis, publishing decisions, or manuscript preparation.

## S3P65. PERCEPTUAL FLUENCY AND COGNITIVE EFFORT IN AUDITORY PROCESSING OF MUSICAL CONTENT REINTERPRETED BY ARTIFICIAL INTELLIGENCE

Radu-Gabriel Boboc, Andreea-Simona Burtoiu, Luiza Dabija, Nicolae Demit, Alina-Catrinel Dodescu (Manolescu), Violeta-Ștefania Rotarescu

*Department of Applied Psychology and Psychotherapy, University of Bucharest, Bucharest, Romania*

INTRODUCTION: Perceptual fluency, defined as the subjective ease with which a stimulus is cognitively processed (Reber et al., 1998), is a predictor of processing effort. Previous research has shown that stimuli with familiar structures are processed with reduced cognitive effort and shorter reaction times (Winkielman & Cacioppo, 2001), though most studies have focused on neutral or positive stimuli.

AIM(S): The present study (N=31, within-subjects) investigates the role of perceptual fluency in the pro-

cessing of auditory stimuli with aversive content, using AI-generated musical reinterpretations.

METHOD(S): Participants who declared an aversion to modern fiddle music are exposed to both fragments of the original songs and fragments of the songs in the AI-reinterpreted version of the same songs, in a blues/jazz style. Perceptual fluency is measured as reaction time to lyrics comprehension, message evaluation, and source attribution accuracy (AI *vs.* human interpreter).

RESULTS: The results show that familiar musical structure, as interpreted by AI, reduces reaction time and leads to more weighted evaluations of the message, even in the presence of aversive semantic content. In addition, the results show a low sensitivity in identifying the source of reinterpretation.

CONCLUSIONS: The results may contribute to the understanding of cognitive mechanisms by which the perceptual structure of the auditory stimulus (rhythm, harmony, timbre) influences the processing of aversive semantic content and to the understanding of literature on the perception of content generated by artificial intelligence.

FINANCIAL SUPPORT: Self-supported research.

## S3P66. STRATEGIC MIND WANDERING AND THOUGHT CONTENT IN NATURAL LISTENING

Michał Domagała[1], Marcin Leszczyński[1,2]

[1] *Cognitive Science Center, Jagiellonian University, Kraków, Poland*

[2] *Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York City, USA*

INTRODUCTION: Mind wandering (MW) refers to a shift in attention away from the task at hand toward internally generated thoughts. While traditional accounts conceptualize MW as a failure of executive control, resource-based theories propose it reflects strategic allocation of cognitive resources when external task demands are low. Evidence for such modulation has largely come from controlled, semantically and temporally impoverished studies.

AIM(S): We aimed to characterize how the content and temporal dynamics of reported experience change during MW in continuous, meaningful contexts. Specifically, we tested whether MW is strategically expressed during task-irrelevant (uncued) segments of a naturalistic narrative, and whether semantic coupling between verbal reports and the narrative decreases during these periods.

METHOD(S): Participants listened to a custom-designed auditory narrative alternating between two protagonists and were instructed to later recall information about one (cued) while ignoring the other (uncued). After each segment, they reported their attentional state using experience-sampling probes. In a second experiment, participants also provided verbal descriptions of their ongoing experience. Eye movements and pupil diameter were recorded. Using language processing methods (sBERT and word2vec embeddings) we quantified semantic coupling between verbal reports and the narrative.

RESULTS: We observed more frequent MW during uncued compared to cued segments. This pattern replicated across self-reports and independent analysis of their verbal reports. Semantic analyses revealed reduced coupling between the narrative and participants' expressed content during uncued segments and MW episodes.

CONCLUSIONS: These findings demonstrate that MW can be strategically modulated in semantically rich environments. MW was also associated with reduced coupling to ongoing input, extending the laboratory-based accounts of strategic MW to continuous narrative comprehension.

FINANCIAL SUPPORT: This research was funded by a grant from National Science Center of Poland (Sonata 19: UMO-2023/51/D/HS6/02920).

## S3P67. DYNAMIC ENGAGEMENT OF INHIBITORY CONTROL IN SEMANTIC MEMORY RETRIEVAL

Veronika Zelenayová[1], Adam Kubinec[1], Martin Marko[1,2]

[1] *Department of Behavioural Neuroscience, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovak Republic*

[2] *Department of Applied Informatics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Bratislava, Slovak Republic*

INTRODUCTION: Inhibition is a core executive function enabling flexible behavior by suppressing inappropriate responses and thoughts. Converging evidence indicates that it may also regulate memory processes, yet the mechanisms underlying this role remain unclear.

AIM(S): In two experiments, we investigated the neurocognitive mechanisms of inhibitory control by dissociating whether and how inhibition proactively prevents or reactively suppresses irrelevant conceptual activation in memory.

METHOD(S): The first experiment (N=75) employed a novel paradigm manipulating the requirement for inhibitory control during semantic retrieval (free-associative *vs.* dissociative/inhibitory task) and its timing (baseline *vs.* pre-cued trials), while assessing retrieval latency and associative intrusions. This was followed by old/new recognition test assessing memory for stimuli presented during the main task. The second experiment (N=31) combined this paradigm with pupillometry, enabling time-resolved analysis of pupil dilation across the retrieval tasks to characterize the temporal dynamics of inhibitory engagement.

RESULTS: Although pre-cuing improved retrieval performance, this benefit was attributable to facilitated task switching rather than inhibition. More importantly, efficient inhibitory engagement during retrieval predicted impaired recognition performance, suggesting that inhibition is recruited early to attenuate depth of stimulus processing (encoding) and thereby limit the activation of related but interfering memory representations. This interpretation was further supported by pupillometric analyses revealing two temporally dissociable, antagonistic components: an early component consistent with inhibitory engagement and a later component consistent with interference resolution.

CONCLUSIONS: Together, these findings provide evidence for an early-acting inhibitory mechanism that adaptively attenuates processing depth of incoming stimuli to reduce retrieval interference.

FINANCIAL SUPPORT: APVV-23-0145, VEGA 2/0052/23.

## S3P68. ULTRA SHORT HRV AS AN AUTONOMIC MARKER FOR NEUROCOGNITIVE EXPERIMENTS: VALIDATION ACROSS RESTING STATE CONDITIONS IN A PLACEBO CONTROLLED CAFFEINE STUDY

Cezary Zając[1], Karina Maciejewska[2], Klaudia Duch[2], Maciej Giza[3], Aleksandra Nas[3]

[1] *College of Interdisciplinary Individual Studies, University of Silesia in Katowice, Katowice, Poland*

[2] *Faculty of Science and Technology, Institute of Biomedical Engineering, University of Silesia in Katowice, Chorzow, Poland*

[3] *Radiotherapy Planning Department, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Gliwice, Poland*

INTRODUCTION: Heart rate variability (HRV) is a widely used index of autonomic nervous system activity that complements central measures in cognitive neuroscience by tracking arousal, parasympathetic regulation, and sympathovagal balance. Current clinical guidelines recommend at least 5 min of ECG for short term HRV analysis, limiting use in trial based designs and dynamic paradigms where psychophysiological state changes rapidly.

AIM(S): We aimed to validate ultra short HRV (ustHRV) measures against the 5 min benchmark across multiple recording lengths and psychophysiological conditions.

METHOD(S): We analyzed ECG from 47 participants in a double blind placebo controlled study assessing an energy supplement containing caffeine versus matched control. Recordings were acquired during eyes-open and eyes-closed resting states at three time points: before supplementation, 30 min, and 90 min after. Three time domain (RMSSD, SDSD, pNN50) and five frequency domain indices (HF power, LF power, normalized HF, normalized LF, LF to HF ratio) were computed from 10 s, 30 s, 1 min, 2 min, 3 min, and 4 min segments and compared with 5 min reference values.

RESULTS: Across correlation analyses, intra and inter group tests, trend analyses, and Bland Altman agreement, time domain indices and HF power from 30 s segments showed good concordance with 5 min benchmarks, whereas other frequency measures generally required 1 to 2 min segments. Our findings align with HRV electrophysiology: slower frequency measures are more sensitive to state changes such as mental fatigue. Eyes open versus closed conditions did not substantially alter optimal segment length when recordings were brief, but extended sessions inducing sleepiness or fatigue may require longer ECG to preserve agreement.

CONCLUSIONS: These results support ustHRV as a feasible autonomic marker for neurocognitive studies with constrained time windows, while highlighting that optimal segment length should match expected state stability during tasks rather than being a fixed rule.

FINANCIAL SUPPORT: The research activities were co-financed by the funds granted under the Research Excellence Initiative of the University of Silesia in Katowice.

## S3P69. HOW BRAIN ACTIVITY CHANGES WHEN WE STOP PROCRASTINATING: A RANDOMIZED CONTROLLED FMRI STUDY OF TREATMENT MECHANISMS IN CBT FOR PROCRASTINATION

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

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

[2] *Poznań Laboratory of Affective Neuroscience, Institute of Psychology, SWPS University, Warsaw, Poland*

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

INTRODUCTION: Procrastination is a common self-regulatory failure characterized by an irrational delay of personally important but aversive tasks, and is often linked to impaired well-being. Although cognitive-behavioral therapy (CBT) is regarded as the most promising treatment for procrastination, neural mechanisms underlying its effectiveness remain unexplored. Prior fMRI research indicates that highly procrastinating individuals show reduced recruitment of prefrontal control regions – particularly the anterior cingulate cortex (ACC) and right dorsolateral prefrontal cortex (dlPFC) – when performing cognitive tasks in emotionally negative or punitive contexts, possibly reflecting behavioral avoidance and impaired processing of negative consequences. These findings suggest that attenuated activity in prefrontal control regions may underlie chronic procrastination and could serve as a target for interventions.

AIM(S): In this randomized controlled trial, we examined how procrastination treatment affects brain activity using longitudinal task-based fMRI.

METHOD(S): Data were collected from 112 help-seeking, high-procrastinating university students before and after a 5-week group CBT intervention or a wait-list control period. The intervention effectively reduced self-report procrastination (Aitken Procrastination Inventory–Revised) compared to the control group (d=-0.68). During fMRI scanning, participants completed a Go/No-Go task with two conditions: a monetary punishment condition, in which money was deducted for errors or slow responses, and a neutral condition, where no rewards or penalties were applied.

RESULTS: Neuroimaging analyses are currently in progress and results will be presented at the conference.

CONCLUSIONS: Based on prior evidence, we hypothesize that participants who received CBT will show enhanced activation in prefrontal control regions (i.e., ACC, right dlPFC) during the punishment condition, reflecting improved self-regulation and cognitive control in emotionally negative contexts.

FINANCIAL SUPPORT: This research was conducted by the GRAPPA consortium (General Research Assessing Psychotherapy for Procrastination Applications) and funded by the Polish National Science Centre grant 2021/43/B/HS6/02024 awarded to MW, JMM and JK.

## S3P70. REDUCED AROUSAL FOLLOWING SLEEP DEPRIVATION DOES NOT DIMINISH PSEUDONEGLECT IN FREE VISUAL EXPLORATION

Aleksandra Smus[1], Wiktor Więcławski[1,2], Wojciech Żukowski[1], Marek Binder[1]

[1] *Institute of Psychology, Jagiellonian University, Kraków, Poland*

[2] *Doctoral School of Social Sciences, Jagiellonian University, Kraków, Poland*

INTRODUCTION: When freely exploring naturalistic scenes, healthy individuals typically exhibit an initial attentional bias toward the left visual field. Although this phenomenon—known as pseudoneglect—is robust and easily replicable, its underlying mechanisms remain unclear. Beyond explanations invoking hemispheric asymmetries in attentional control, it has been proposed that arousal plays a key role in modulating the magnitude and direction of this bias. Several studies have suggested that reduced arousal may attenuate leftward bias or even lead to a reversal toward the right visual field.

AIM(S): Given the lack of a clear mechanistic account and mixed empirical evidence, replication of these findings is warranted. We also aimed to disentangle the effects of two main drivers of low arousal: circadian rhythms and sleep deprivation.

METHOD(S): We therefore conducted an experiment in 42 right-handed young adults to examine whether reductions in arousal influence pseudoneglect during free visual exploration. Participants performed a free-viewing task under three conditions: normal arousal in the evening (20:00), reduced arousal at the circadian trough (05:00), and following a full night of sleep deprivation (09:00). Arousal and vigilance were carefully monitored using behavioral measures.

RESULTS: As expected, arousal levels were lowest at 05:00. Despite this reduction, visual exploration patterns did not differ across conditions, with all sessions showing a robust and comparable leftward bias.

CONCLUSIONS: These findings suggest that transient reductions in arousal, including those induced by circadian phase and sleep deprivation, do not diminish pseudoneglect. Our results contribute to the ongoing debate on the mechanisms underlying pseudoneglect and indicate that factors other than global arousal may play a more critical role in shaping spatial attentional biases.

FINANCIAL SUPPORT: The study was supported by the grant awarded by Rada Kół Naukowych Uniwersytetu Jagiellońskiego to Aleksandra Smus (RKN/2025/2A/020).

## S3P71. DISTINCT ALTERATIONS IN SOCIAL SEMANTIC PROCESSING ARE LINKED TO ALEXITHYMIA AND AUTISM TRAITS

Marcos Ibáñez Montolio[1], Iván González-García[1], Richard J. Binney[2], Paul Hoffman[3], Michal Rafal Zareba[1], Maya Visser[1]

*[1] Neuropsychology and Functional Neuroimaging Group, Department of Basic and Clinical Psychology and Psychobiology, Jaume I University, Castelló de la Plana, Spain*

*[2] Department of Psychology and Bangor Imaging Unit, Bangor University, Gwynedd, United Kingdom*

*[3] School of Philosophy, Psychology & Language Sciences, University of Edinburgh, Edinburgh, United Kingdom*

INTRODUCTION: Alexithymia is a multifaceted and dimensional condition characterized by difficulties identifying and describing feelings, and a decreased ability to engage in abstract thinking. Although an independent construct, it often co-occurs with autism spectrum disorders, in which it is believed to underlie the observed emotion processing difficulties. Converging lines of evidence point towards the key role of semantic cognition in socio-emotional processing, and the importance of serotonin, dopamine and noradrenaline, in enabling socially-driven emotions and cognitions. Accordingly, we expect alexithymia and autism traits to produce unique alterations in these networks underlying socio-emotional cognition.

AIM(S): The current study aimed to elucidate this matter by investigating how alexithymia and autism facets were associated with brain activity and connectivity patterns during two tasks probing distinct aspects of social cognition using a large sample of 126 healthy adults.

METHOD(S): Task connectivity analyses focused on the interactions of the brain areas involved in the social semantics networks, specifically the anterior temporal lobes, and the brainstem areas behind the brain's neuromodulatory responses, i.e. dorsal raphe, ventral tegmental area, and locus coeruleus.

RESULTS: We observed significant associations for both task activity and connectivity, with the majority of effects found for the latter. Specifically, across both tasks, alexithymia was to a greater extent associated with functional connectivity between seed regions and brain areas involved in pain somesthesis, visual perception and action inhibition, while autism results were more strongly related with areas involved in explicit and working memory, language and social cognition.

CONCLUSIONS: Collectively, these findings suggest that both constructs are associated with alterations in areas related to distinct cognitive processes, highlighting the need to distinguish between them in both research and clinical settings.

FINANCIAL SUPPORT: Research projects awarded to Maya Visser: Grant PID2021-127516NB-I00 funded by MICIU/AEI/10.13039/501100011033 and by "ERDF/EU", Grant RYC2019-028370-I funded by MICIU/AEI/10.13039/501100011033 and by "ESF Investing in your future", Grant CIAICO/2021/088 funded by Conselleria de Educación, Universidades y Empleo and Grant UJI-B2022-55 funded by Universitat Jaume I.

## S3P72. ALEXITHYMIA AND EMOTIONAL AROUSAL IN WOMEN WITH NON-SUICIDAL SELF-INJURY – A COMPARATIVE ANALYSIS

Julia Lasota, Helena Turek

*Department of Biological Psychology, Center for Behavioral Neuroscience, Faculty of Psychology, SWPS University of Social Sciences and Humanities, Warsaw, Poland*

INTRODUCTION: Non-Suicidal Self-Injury (NSSI) is the deliberate harm to one's body without suicidal intent, often used to regulate intense negative emotions. Alexithymia involves difficulties identifying and describing emotions, leading to impaired emotion regulation. Exploring links between emotional arousal, alexithymia, and NSSI may clarify mechanisms of self-injury.

AIM(S): The aim of this study was to investigate the relationship between alexithymia and emotional arousal in women engaging in Non-Suicidal Self-Injury (NSSI). Specifically, the study sought to compare emotional reactivity and affective valence in response to different types of visual stimuli, as well as levels of alexithymia, between women with a history of NSSI and a control group.

METHOD(S): The study involved 405 adult women aged 18 to 22, including 214 individuals with a history of NSSI and 191 women in the control group. Among the entire sample, 209 participants displayed alexithymic traits. The following research instruments were used: the Inventory of Statemets About Self-Injury (ISAS), the Toronto Alexithymia Scale (TAS-20), and a custom-developed computer program designed to assess emotional arousal and affective valence.

RESULTS: Women with NSSI did not differ from controls in emotional arousal, but they reported lower negative affect in response to drastic and negative images. They showed higher levels of alexithymia, which was positively associated with difficulties in identifying and verbalizing emotions. The most commonly reported functions of self-injury were affect regulation and self-punishment.

CONCLUSIONS: Women engaging in non-suicidal self-injury (NSSI) showed similar emotional arousal to controls but lower negative affect to extreme negative images. They had higher alexithymia, with NSSI linked to difficulty identifying/verbalizing emotions. NSSI served mainly affect regulation and self-punishment. Responses to positive stimuli highlight the potential of boosting positive emotions in therapy.

FINANCIAL SUPPORT: none.

## S3P73. PSILOCIN MODULATE THE MICROGLIA ACTIVITY IN EXPERIMENTAL MODEL OF NEUROINFLAMMATION EVOKED BY LIPOPOLYSACCHARIDE TREATMENT

Kinga Kaim[1], Monika Leskiewicz[2], Magdalena Regulska[2], Ewa Trojan[2], Krystyna Gołembiowska[1]

[1] *Department of Pharmacology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Department of Experimental Neuroendocrinology, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

INTRODUCTION: Microglia are the primary resident immune cells of the central nervous system that are responsible for the maintenance of brain homeostasis. Among others they are capable, for nitric oxide (NO) synthesis and various cytokines release. A plethora of evidence suggest that microglia display distinct phenotypes that are associated with the alteration of cell activation under varying environmental cues. Psilocin is the bioactive metabolite of the naturally occurring indole tryptamine – psilocybin, which beneficial effects for some brain disorders treatment was reported. However the precise mechanisms underlying therapeutic effects remain only partially understood.

AIM(S): The objective of this study was to assess the effect of psilocin on the chosen parameters of microglial activity in basal conditions and after lipopolysaccharide (LPS) stimulation.

METHOD(S): Primary microglia cultures were prepared from cortices of 1-2-day-old offspring. Psilocin (10 μM) was added for 1 hour, then microglia were exposed to lipopolysaccharide (LPS, 100 ng/ml) for 24 hours. Cell death was determined by the LDH test. Nitric oxide (NO) synthesis was assessed by the Griess reaction. Protein levels of various cytokines release, A20 (a negative regulator of NF-kB) and the phosphorylation level of the p65 NF-κB subunit were measured using ELISA kits.

RESULTS: Administration of psilocin resulted in a decrease in NO production and mortality of the LPS-stimulated microglia cells. Moreover, psilocin diminished evoked by LPS pro-inflammatory cytokines (IL-1b, TNF-a, IL-6) release. This mechanism was related with decrease in the phospho-p65/total p65 ratio and normalization of the LPS-induced decrease in A20 levels.

CONCLUSIONS: The results indicate that psilocin may have an important influence on microglia cell activation. The intricate mechanisms by which psychedelics modulate neuroinflammation underscores their potential as innovative therapeutic agents for treating inflammatory-related brain disorders.

FINANCIAL SUPPORT: This work was supported by statutory funds of the Department of Experimental Neuroendocrinology PAS and National Science Centre grant no. 2020/37/B/NZ7/03753.

www.ane.pl