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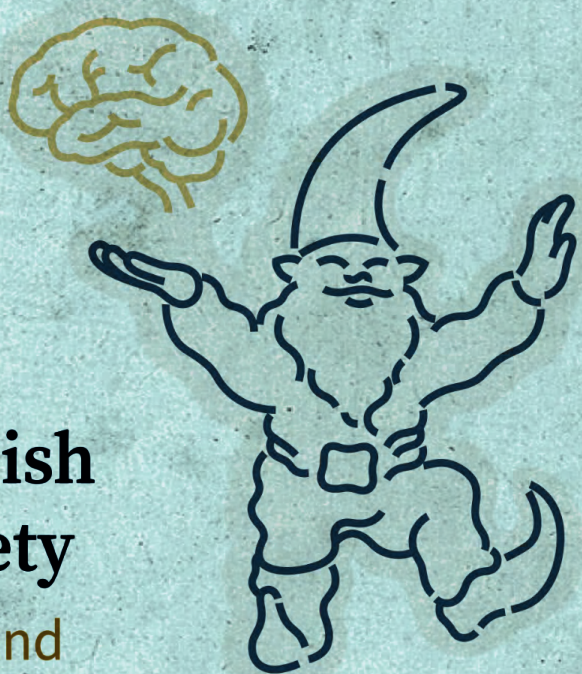

17th International Congress of the Polish Neuroscience Society

2-5.09.2025 Wrocław, Poland

NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY, WARSAW, POLAND

17th INTERNATIONAL CONGRESS OF THE POLISH NEUROSCIENCE SOCIETY

2-5.09.2025 WROCŁAW, POLAND

CONTENTS

WELCOME

We are delighted to welcome the neuroscience community to the 17th International Congress of the Polish Neuroscience Society (PTBUN)!

Founded in 1991, PTBUN has played a pivotal role in uniting researchers from diverse branches of neuroscience and promoting international cooperation. Regular Congresses serve as a forum for scholars to share exciting discoveries, exchange ideas, explore collaboration opportunities, and create novel research alliances. PTBUN dedicated to cultivating an environment encouraging interdisciplinary dialogue and stimulating interactions with discipline leaders worldwide.

For the first time, the Congress is hosted by Wrocław, the heart of Lower Silesia, marked by a complex history and multinational heritage. Wrocław has long traditions in scientific excellence, with more than 10 Nobel prize winners born, studied, or lived in the city. The Department of Psychiatry, established in the late 19th century, was led by godfathers of neurology, including Hans Wernicke and Alois Alzheimer. Nowadays, local research capacity is rapidly growth through innovation centers (e.g. Wrocław Biotech Hub called to live in 2025, R&D centers of the world's biggest companies) and emerges as an important landmark on the European map of science & technology.

The Congress is organized jointly by the PTBUN, Łukasiewicz – PORT Polish Center for Technology Development and Wrocław University of Science and Technology, with the active contribution of University of Wrocław, Wrocław Medical University, and Wrocław University of Environmental and Life Sciences.

We are encouraging you to benefit from the opportunity to network and build lasting connections with colleagues from diverse disciplines of brain research.

We wish you a memorable and enriching scientific gathering and wonderful time exploring the cultural offerings of Wrocław.

Irena Nalepa
Chair of the Scientific Committee
President of the Polish Neuroscience Society

Michał Ślęzak
Chair of the Organizing Committee
Łukasiewicz Research Network – PORT

ORGANIZERS & PARTNERS

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PTBUN Polish Neuroscience Society

Łukasiewicz PORT

Wrocław University of Science and Technology

PARTNERS AND CO-ORGANIZERS:

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Wrocław the meeting place

Uniwersytet Wrocławski | Patronat Honorowy Rektora Uniwersytetu Wrocławskiego

Wrocław University of Environmental and Life Sciences

PAN Polish Academy of Sciences

International Brain IBRO Research Organization

75 Years Wrocław Medical University

WCA Wrocławskie Centrum Akademickie

NAWA Polish National Agency for Academic Exchange

Fundacja Polskiego Ośrodka Rozwoju Technologii

SAME-NeuroID

Funded by the European Union

SCIENTIFIC COMMITTEE

ORGANIZING COMMITTEE

Chair of the Organizing Committee

dr Michał Ślęzak
Łukasiewicz Research Network – PORT
Polish Center for Technology Development, Wrocław

Event Coordinator

Marta Małolepsza
Łukasiewicz Research Network – PORT
Polish Center for Technology Development, Wrocław

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Wrocław University of Science and Technology

dr Natalia Małek-Chudzik
Wrocław University of Science and Technology

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Maj Institute of Pharmacology PAS, Cracow

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Jagiellonian University, Cracow

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Łukasiewicz Research Network – PORT
Polish Center for Technology Development, Wrocław

Sandra Markwart-Socha
Łukasiewicz Research Network – PORT
Polish Center for Technology Development, Wrocław

Paulina Macierzyńska
Łukasiewicz Research Network – PORT
Polish Center for Technology Development, Wrocław

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volunteers involved in the organization of the conference

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Jagiellonian University, Cracow

Gabriela Czerniak
Jagiellonian University, Cracow

Magdalena Siwarga
Jagiellonian University, Cracow

Martyna Bernaciak
Jagiellonian University, Cracow

Emil Wawak
Jagiellonian University, Cracow

Dana Ospanova
Jagiellonian University, Cracow

Dobrawa Świder
University of Wrocław

Patryk Betlej
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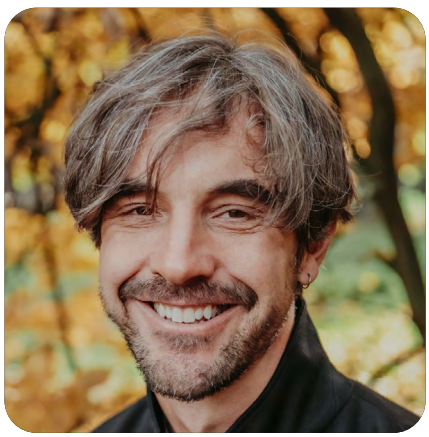
KEYNOTE SPEAKERS



MARCIN SZWED

Marcin Szwed is a neuroscientist. He studied biology at the Jagiellonian University. After completing his PhD at the Weizmann Institute and spending five years as a postdoctoral researcher in Paris, he returned to Cracow in 2011, where he established his own research team focused on human brain imaging. His research focuses on change in the brain in its both positive and negative aspects.

The positive change is the plasticity that occurs when the brain is reorganized in individuals who are blind or deaf. It is a process where a part of the brain, for example the visual cortex, is either rewired to perform an old task, such as reading, with a new sense, such as touch, or rewired to perform a new task, like language or memory. Deaf and blind people have lost critical sensory input and this has pro- foundly altered the way their brains work. By studying them, we can understand the forces that shape the brain.

The negative change that we study is the detrimental impact of air pollution on brain development. Since 2019, he has begun his journey into environmental neuroscience as the leader of the NeuroSmog project, which aims to investigate the impact of air pollution on the developing brains of school-aged children. While the harmful effects of smog on respiratory and cardiovascular diseases are well known, much less is understood about its effects on the brain. Fighting smog has a social cost. If we ask people to make sacrifices and change their lifestyles, we must obtain the best possible knowledge about the impact of this pollution. This is the purpose of the NeuroSmog project.

Prof. Szwed has published several dozen scientific articles, including a few that he considers truly important. He has received several awards.

NANCY M. BONINI

Dr. Bonini is Professor of Biology at the University of Pennsylvania in Philadelphia, PA. After receiving her PhD at the University of Wisconsin-Madison, she performed postdoctoral work at Caltech learning the Drosophila system, in studies with Dr. Seymour Benzer.

She started her own research laboratory at Penn in 1994, where she launched the studies that use Drosophila as a model for human disease. In this work, she expressed various human disease genes in the fly to show that these genes mimicked human neurodegenerative disease features, then launched discovery genetic pathways that influence the effects. She is currently the Florence RC Murray Professor of Biology, and has been elected to the National Academy of Sciences, the National Academy of Medicine and the American Academy of Arts and Sciences.

LESZEK KACZMAREK

Leszek Kaczmarek is Professor at the Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland, head of the Laboratory of Neurobiology and President of the Center of Excellence for Neural Plasticity and Brain Disorders: BRAINCITY; a Nencki-EMBL Partnership.

Leszek Kaczmarek has got his PhD in experimental hematology (mentor: Prof. W. Wiktor-Jedrzejczak), followed by D.Sc. (dr hab.) in the field of experimental oncology. He carried out postdoctoral studies in Philadelphia, USA (mentor: Prof. R. Baserga) and then was visiting professor in the University of Catania, Italy; McGill University, Montreal, Canada; University of California, Los Angeles and Institute of Photonic Sciences, ICFO, Castelldefels, Spain. Since 1986 his laboratory at the Nencki Institute has been investigating brain-mind connection at all the levels of brain organization from molecular to cellular to network to behavior in health and disease. Most of the work involves experimental animal models, however joint studies with clinicians on human neuropsy-

chiatric disorders have also been pursued. The current major focus is on extracellular enzyme, matrix metalloproteinase, MMP-9, which his laboratory documented to play paramount role in neuronal/ synaptic plasticity and then in learning and memory, development of epilepsy, schizophrenia, autism spectrum disorders and alcohol addiction.

In his recent studies, prof. Kaczmarek and his team, aimed to test whether MMP-9 might be engaged in epileptogenesis (epilepsy development). Indeed, they have provided a genetic proof (by using MMP-9 gene knockout to impair epileptogenesis and MMP-9 over-expressing rats and mice to enhance the epilepsy development) supporting that notion. To follow up on those results, they have recently tested inhibitors of MMP-9 enzymatic activity as therapeutics to prevent epileptogenesis in such clinically relevant preclinical models, as traumatic brain injury and stroke in mice. In result, they have identified MMP-9 inhibitor as a possible anti-epileptogenesis drug that is currently being prepared for phase one clinical trials.

LORA HEISLER

Professor Lora Heisler is Chair in Human Nutrition and Director of Research of the Rowett Institute, University of Aberdeen, Scotland. Professor Heisler began her independent research group at Beth Israel Deaconess Medical Center & Harvard Medical School and then relocated to the University of Cambridge, UK.

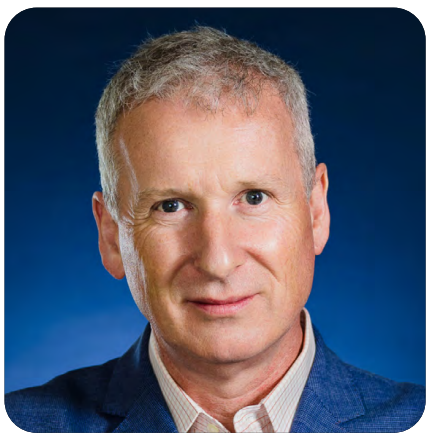
Her group moved to the Rowett Institute in 2013. Professor Heisler's career contributions to obesity and diabetes research were acknowledged by Outstanding Scientific Achievements Awards from the Obesity Society and American Diabetes Association. She was elected to Scotland's national academy of science and letters The Royal Society of Edinburgh in 2016. Professor Heisler's research focuses on the brain circuits underlying appetite, physical activity, body weight and glucose homeostasis in an effort to identify new targets amenable for obesity and type 2 diabetes treatment.

ANDREW HOLMES

Andrew Holmes specializing in neuroscience, with a focus on behavior and addiction. He was trained in the UK, where received his Bachelor's (Hons) degree in Psychology and his Doctorate in Behavioral Pharmacology. He received postdoctoral training in behavioral neuroscience and behavioral genetics from Dr. Jacki Crawley at the NIMH.

He was recruited to the NIAAA in 2004 and is Currently leading the Laboratory of Behavioral and Genomic Neuroscience at the National Institute on Alcohol Abuse and Alcoholism (NIAAA), part of the National Institutes of Health (NIH), where he studies brain regulation of emotion and cognition using animal models. He obtained numerous awards, including in 2022 the NIAAA Scientific Achievement Award for 'scientists who have made an outstanding contribution to scientific research'. The mission of the Laboratory of Behavioral and Genomic Neuroscience is to contribute to a deeper understanding of the causes of alcoholism and comorbid neuropsychiatric conditions such as mood and anxiety disorders.

Our goal is to help identify new directions for the prevention and effective treatment of these devastating diseases. To this end, we are using models of chronic alcohol exposure and chronic stress to examine how these environmental insults reshape brain circuits to modify behavior, and why they do so in a manner that varies greatly from individual to individual as a function of genetics, sex and age. A major current focus of our work is how alcohol and stress affect the structure and function of circuits interconnecting the prefrontal cortex with limbic and dorsal striatal regions that are critical for the regulation of emotion, cognition and executive control over drug-seeking.



JOHN J. FOXE

John J. Foxe, PhD, is director of both the Ernest J. Del Monte Institute for Neuroscience and the newly formed Golisano Intellectual and Developmental Disabilities Institute at The University of Rochester. His research investigates the neurobiological bases of neurodevelopmental and neuropsychiatric conditions such as autism and schizophrenia.

He uses electrophysiological and neuroimaging techniques to understand how inputs from the various sensory systems are combined in the brain, and what happens when these multisensory integration abilities are impacted by disease.

Foxe has authored more than 350 research and clinical papers, book chapters, commentaries, and proceedings and serves as editor-in-chief of The European Journal of Neuroscience.

JOHN F. CRYAN

John F. Cryan is Professor & Chair, Dept. of Anatomy & Neuroscience, University College Cork (UCC) and has been Vice President for Research & Innovation since 2021. He is also a Principal Investigator in the APC Microbiome Institute.

He received a B.Sc. (Hons) in Biochemistry and PhD in Pharmacology from the University of Galway, Ireland and was a visiting fellow at the Dept Psychiatry, University of Melbourne, Australia, which was followed by postdoctoral fellowships at the University of Pennsylvania, Philadelphia, USA and The Scripps Research Institute, La Jolla, California. He spent four years as a Group Leader in the pharmaceutical industry with Novartis in Basel Switzerland prior to joining UCC in 2005. Prof. Cryan's current research is focused on understanding the interaction between brain, gut & microbiome and how it applies to stress, psychiatric and immune-related disorders at key time-windows across the lifespan. Prof. Cryan has published over 700 peer-reviewed articles, co-edited four books and is co-author of the bestselling „The Psychobiotic

Revolution: Mood, Food, and the New Science of the Gut-Brain Connection". He has received numerous awards including from UCC, the University of Utrecht, University of Antwerp, American Gastroenterology Association, Neuroscience Ireland, Neonatal Society, European College of Neuropsychopharmacology, British Association of Psychopharmacology, Physiological Society, Royal Academy of Medicine in Ireland, & FASEB. He has been named on the Clarivate Highly Cited Researcher each year since 2017. He was elected a Member of the Royal Irish Academy in 2017 and is Past-President of the European Behavioural Pharmacology Society. He has been a TEDMED and TEDx speaker and was profiled in the Netflix documentary Hack Your Health: The Secrets of the Gut in 2024.

FLATAU AWARD

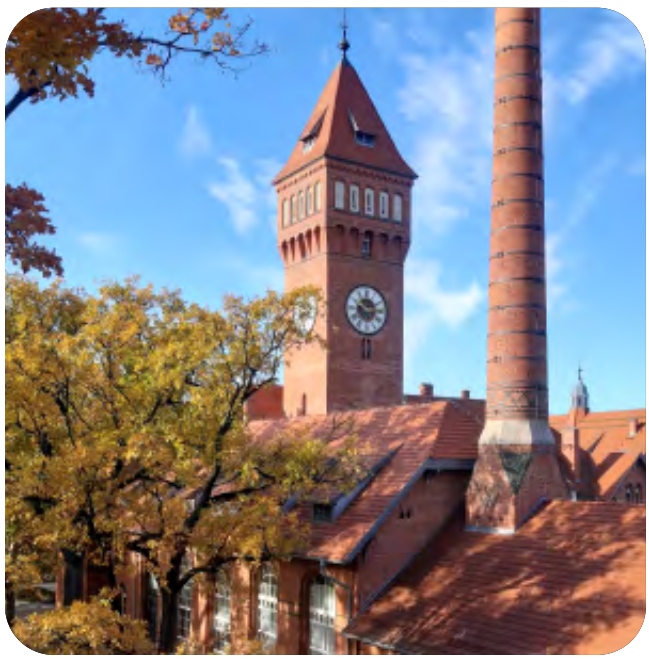
PIOTR MAJKA

Dr. Piotr Majka, Assistant Professor at the Nencki Institute of Experimental Biology at the Polish Academy of Sciences, leads a research team focused on understanding how the brain's intricate microscopic connectivity network influences perception, behavior, and actions.

His interdisciplinary approach integrates high-throughput methods for analyzing microscopic brain images with computational modeling, machine learning, and artificial intelligence to conduct in-depth data analysis. Dr. Majka also incorporates classical neuroanatomy and neurophysiology to ensure a robust neurobiological foundation for his research, emphasizing the principles of open science.

With 15 years of experience, Dr. Majka has made significant contributions to neuro-informatics, including the development of multimodal 3D brain atlases, advanced image registration techniques, and neuroinformatics platforms. His work has produced the most comprehensive cortico-cortical connectome of any non-human primate brain to date. Additionally, Dr. Majka has held positions as an Adjunct Research Fellow at Monash University in Melbourne, Australia, and as a Visiting Research Scientist at the Neuroinformatics Japan Center, RIKEN Center for Brain Science in Wako, Japan. Dr. Majka's scientific achievements were recognized with the Polish Prime Minister's Award for outstanding scientific achievements in 2024.

ŁUKASIEWICZ — PORT



Łukasiewicz – PORT is a modern research and development center in Wrocław, part of the Łukasiewicz Research Network – one of the largest research networks in Europe. We conduct interdisciplinary scientific research and, together with business partners, seek new technological opportunities and innovative solutions for today's pressing problems. Our activities focus on health, biotechnology, and materials engineering.

Łukasiewicz – PORT is home to three research centers: **the Center for Population Diagnostics, the Center for Materials Engineering, and the Center for Life Sciences and Biotechnology**, as well as the Institute's **Center of Laboratories**. In 18 research groups, 160 scientists from Poland and abroad are currently carrying out over 50 research projects with a total value exceeding PLN 270 million.

One of our most important projects is **P4Health: Centre of Excellence for Precise Phenotyping and BioDataBanking**, implemented under the Horizon Europe and Horizon WIDERA programs. This groundbreaking initiative combines biotechnology, bioengineering, and artificial intelligence: across five technology platforms, we are developing AI algorithms for the analysis of genetic and protein data to support the diagnosis and treatment of oncological and neurological diseases. The cornerstone of the project is the development of the biobank at Łukasiewicz – PORT and the promotion of the biobanking concept in Poland.

Our goal is to create the solutions necessary to implement personalized medicine and facilitate its use in everyday medical practice.

Securing funding for P4Health would not have been possible without our earlier Horizon Europe Twinning project – **SAME-NeuroID** – dedicated to standardizing protocols for modeling neurobiological parameters associated with neuropsychiatric disorders, one of the most serious problems in modern society. The Satellite Symposium "European Networking for Brain Research", organized within the framework of the 17th PTBUN Congress, is the main dissemination event of SAME-NeuroID. It provides a dedicated forum for presenting and discussing the project's key findings, bringing together partners and leading experts from across Europe to share results, exchange experiences, and explore next steps in collaborative brain research. The programme presents key project outcomes and strategic discussions on Poland's access to European research networks. Combining scientific presentations with strategic discussions, it strengthens links between Polish and European brain research communities and ensures the project's impact continues.

More information at: **port.lukasiewicz.gov.pl**

PROGRAMME

DAY 1
2nd SEPTEMBER 2025

from 08.00 **Registration Opens**

10.00 – 13.30 **Satellite Symposium: European Networking for Brain Research** [Hall 10 A/C]

10.00 – 11.00 **HE Twinning 'SAME-NeuroID'** [Hall 10 A/C]
Chair: **Witold Konopka** (Łukasiewicz – PORT, Wrocław, Poland)
Speakers: **Witold Konopka**, **Agnieszka Krzyżosiak**, **Michał Ślęzak**, **Femke de Vrij**, **Mathias Schmidt**

11.00 – 12.00 **HE Pathways to Synergies 'PANERIS'** [Hall 10 A/C]
Chair: **Jan Rodriguez Parkitna** (Maj Institute of Pharmacology, PAS, Cracow, Poland)
Speakers: **Jan Rodriguez Parkitna**, **Toni Andreu**, **Osvaldas Rukšėnas**

12.00 – 12.30 **Coffee Break**

12.30 – 13.30 **Panel Discussion: European Brain Research Initiatives** [Hall 10 A/C]
Moderator: **Dr Monika Ślęzak** (Industry Contact Point Medical Technologies and Health, Łukasiewicz – PORT, Wrocław, Poland)
Panel Guests: **Izabela Najda-Jędrzejewska** (Deputy Director of the Department of Innovation and Development, Polish Ministry of Science and Higher Education (MNiSW)), **Maria Śmietanka** (Deputy Director of National Contact Point in NCBR (KPK)), **Toni Andreu** (Scientific Director, EATRIS), **Cezary Mazurek** (eBRAINS, Director of Poznań Supercomputing and Networking Center), **Jacek Jaworski** (International Institute of Molecular and Cell Biology in Warsaw), **Leonora Bużańska** (Mossakowski Medical Research Institute, PAS, Warsaw, Poland), **Johanna Kostenzer** (Deputy Head Public Affairs, BBMRI-ERIC)

8.30 – 14.00 **Arduino Workshop** [Room 115]

13.30 – 14.15 **Lunch**

14.15 – 17.00 **General Assembly of the Polish Neuroscience Society** [Hall 10 D]

15.00 – 16.45 **Panel Discussion for General Audience** [Hall 10 A/C]
Moderator: **Dr Marta Duda-Sikuła** (Industry Contact Point Medical Technologies and Health, Łukasiewicz – PORT, Wrocław, Poland)
Panel Guests: **Prof. Joanna Rymaszewska** (Faculty of Medicine, Wrocław University of Science and Technology), **Dr Agnieszka Krzyżosiak** (Łukasiewicz Research Network – PORT), **Małgorzata Calińska-Mayer** (Councilor of the Lower Silesian Voivodeship Assembly, Chairwoman of the Lower Silesian Voivodeship Seniors' Council), **Dr Daniel Wójcik** (Wrocław Alzheimer's Center, Lower Silesia Alzheimer's Foundation), **Urszula Kielar** (President of the People of Autumn Foundation), **Dorota Feliks** (Director of the Wroclaw Center for Social Development (Wrocław City Hall)), **Przemysław Matyja** (City Urban Planner, Director of the Department of Spatial Planning (Wrocław City Hall)), **Dr Anna Serweta-Pawlik** (Academy of Physical Education Wrocław)

17.00 – 17.30 **Coffee Break**

17.30 – 18.40 **Official Opening Ceremony Jerzy Konorski Memorial Lecture** [Hall 10 A/C]
Molecular biology of synaptic plasticity
Chair: **Jacek Jaworski** (International Institute of Molecular and Cell Biology, Warsaw, Poland)
Speaker: **Leszek Kaczmarek** (Nencki Institute of Experimental Biology, PAS, Warsaw, Poland)

18.40 – 19.15 **Flashtalks – highlights of submitted abstracts** [Hall 10 A/C]
Chairs: **Anna Błasiak** (Jagiellonian University, Cracow), **Michał Ślęzak** (Łukasiewicz – PORT, Wrocław, Poland)

19.30 **Welcome Reception** [Main Hall]

DAY 2
3rd SEPTEMBER 2025

09.00 – 10.00 **Keynote lecture** [Hall 10 A/C]
Gut feelings – Microbiome, Brain and Behaviour Across the Lifespan
Chair: **Paweł Boguszewski** (Nencki Institute of Experimental Biology, PAS, Warsaw, Poland)
Speaker: **John Cryan** (University of Cork, Cork, Ireland)

10.00 – 10.45 **Flatau Award Lecture** [Hall 10 A/C]
Chairs: **Elżbieta Pyza** (Jagiellonian University, Cracow, Poland), **Irena Nalepa** (Maj Institute of Pharmacology, PAS, Cracow, Poland)
Speaker: **Piotr Majka** (Nencki Institute of Experimental Biology, PAS, Warsaw, Poland)

10.45 – 11.15 **Coffee break**

11.15 – 13.15 **Symposium 1** [Hall 10 A/C]
Advancing neuroscience with unbiased methods of automatization of behavioral studies
Chair: **Bartosz Zglinicki** (Łukasiewicz – PORT, Wrocław, Poland)
Speakers: **Aleksandra Badura**, **Adam Brosnan**, **Bartosz Zglinicki**, **Juan Pablo Lopez**

Symposium 2 [Hall 10 B]
Zinc and the Brain: Unlocking Neurobiological Secrets
Chair: **Bernadeta Szewczyk** (Maj Institute of Pharmacology, PAS, Cracow, Poland)
Speakers: **Andreas M. Grabrucker**, **Jerome Ezan**, **Bernadeta Szewczyk**

Symposium 3 [Hall 10 D]
Novel approaches for the PNS targeting and modulation
Chair: **Mateusz Kucharczyk** (Łukasiewicz – PORT, Wrocław, Poland)
Speakers: **Mateusz Kucharczyk**, **Jimena Perez-Sanchez**, **George Goodwin**, **Sara Jager**

13.15 – 13.45 **Lunch break**

13.45 – 15.00 **Poster session I** [Main Hall]

15.00 – 17.00 **Symposium 4** [Hall 10 A/C]
Neurobiology of Early Life Adversity Across the Lifespan and Across Generations
Chair: **Ali Jawaid** (Łukasiewicz – PORT, Wrocław, Poland)
Speakers: **Mathias Schmidt**, **Aniko Korosi**, **Ali Jawaid**, **Weronika Tomaszewska**

Symposium 5 [Hall 10 B]
On the way to Parkinson's disease: molecular, cellular and clinical aspects of prodromal synucleinopathies
Chair: **Michał Węgrzynowicz** (Mossakowski Medical Research Institute, Warsaw, Poland)
Speakers: **Grzegorz Kreiner**, **Ambra Stefani**, **Giorgio Vivacqua**

Symposium 6 [Hall 10 D]
Copper in the brain
Chairs: **Anna Członkowska** (Institute of Psychiatry and Neurology, Warsaw, Poland) and **Susan Gaskin** (Institute of Psychiatry and Neurology, McGill University, Montreal, Canada)
Speakers: **Anna Członkowska**, **Susan Gaskin**, **Tomasz Litwin**, **Petr Dusek**

17.00 – 17.30 **Coffee Break**

17.30 – 18.30 **Keynote lecture** [Hall 10 A/C]
A neuroscientists' journey into environmental neuroscience. What large-scale scale human neuroimaging can tell us about the impact of environment and society on brain and behavior
Chair: **Anna Błasiak** (Jagiellonian University, Cracow, Poland)
Speaker: **Marcin Szwed** (Institute of Psychology, Jagiellonian University, Cracow, Poland)

DAY 3
4th SEPTEMBER 2025

09.00 – 10.00 **Keynote lecture** [Hall 10 A/C]
Time and Punishment: neural circuits shaping the encoding of adversity
Chair: **Ewelina Knapska** (Nencki Institute of Experimental Biology, PAS, Warsaw, Poland)
Speaker: **Andrew Holmes** (National Institute on Alcohol Abuse and Alcoholism, NIH, USA)

10.00 – 10.45 **Konorski Award Lecture talks for best publication 2023 & 2024** [Hall 10 A/C]
Chairs: **Elżbieta Pyza** (Jagiellonian University, Cracow, Poland), **Irena Nalepa** (Maj Institute of Pharmacology, PAS, Cracow, Poland), **Daniel Wójcik** (Nencki Institute of Experimental Biology PAS, Warsaw, Poland)
Speaker: **Magdalena Dziembowska** (Centre of New Technologies, University of Warsaw, Warsaw, Poland)

10.45 – 11.15 **Coffee Break**

11.15 – 13.15 **Symposium 7** [Hall 10 A/C]
Human Brain Development
Chair: **Bogna Badyra** (Nencki Institute of Experimental Biology, PAS, Warsaw, Poland)
Speakers: **Simona Lodato**, **Antonela Bonafina**, **Aleksandra Pękowska**, **Maciej Figiel**

Symposium 8 [Hall 10 B]
EEG/ECoG based functional connectivity neuroimaging in the rat – towards standardization and translation in neuropsychopharmacology
Chair: **Daniel Wójcik** (Nencki Institute of Experimental Biology, PAS, Warsaw, Poland)
Speakers: **Ivana Chrtkova**, **Theodor Doll**, **Jaroslav Láčík**, **Marian Dovgialo**

Symposium 9 [Hall 10 D]
Brain and metabolism
Chairs: **Joanna H. Śliwowska** (University of Life Sciences, Poznań, Poland) and **Monika Kaczmarek** (Institute of Animal Reproduction and Food Research, Olsztyn, Poland)
Speakers: **Silvia Giatti**, **Monika M. Kaczmarek**, **Paloma Collado**, **Joanna H. Śliwowska**

13.15 – 13.45 **Lunch Break**

13.45 – 15.00 **Poster session II** [Main Hall]

11.15 – 13.15 **Symposium 10** [Hall 10 A/C]
Unraveling the Social Brain: how hierarchies and partners, exogenous substances and hormones shape rodents behavior
Chair: **Hanna Trebesova** (Nencki Institute of Experimental Biology, PAS, Warsaw, Poland)
Speakers: **Hanna Hörnberg**, **Hanna Trebesova**, **Marzena Stefaniuk**, **Alan Kania**

Symposium 11 [Hall 10 B]
Sponsor Presentations
Christian Isidro (I.C.Lab); **Tomasz Guz**, **Emil Nowosielski** (Micro Solutions); **Jaroslav Icha** (Bruker); **Nicolas Bonneau** (Inscopix)

Symposium 12 [Hall 10 D]
Intersecting Pathways: Neuroinflammation in Neurodegenerative Disease
Chair: **Natalia Małek** (Wrocław University of Science and Technology, Wrocław, Poland)
Speakers: **Nico Melzer**, **Lidia Sabater**, **Natalia Małek**, **Jakub Frydrych**

17.00 – 17.30 **Coffee Break**

17.30 – 18.30 **Keynote lecture** [Hall 10 A/C]
Oscillatory Brain Activity and the Deployment of Selective Attention
Chair: **Irena Nalepa** (Maj Institute of Pharmacology, PAS, Cracow, Poland)
Speaker: **John J. Foxe** (University of Rochester, Rochester, NY, USA)

19.00 – 21.00 **IBRO 'Meet-Up and Move-Up' networking reception at the Wrocław Town Hall**

DAY 4
5th SEPTEMBER 2025

09.00 – 10.00 **Keynote lecture** [Hall 10 A/C]
New insights into the brain control of food choice and obesity
Chair: **Joanna Śliwowska** (University of Life Sciences, Poznań, Poland)
Speaker: **Lora Heisler** (University of Aberdeen, Aberdeen, UK)

10.00 – 10.45 **Young Investigator Award talks** [Hall 10 A/C]
Chairs: **Irena Nalepa** (Maj Institute of Pharmacology, PAS, Cracow, Poland) and **Michał Ślęzak** (Łukasiewicz – PORT, Wroclaw, Poland)
Speakers: **Martyna Nalepa** and **Aleksandra Skweres** (Laboratory of Molecular Basis of Neurodegeneration, Mossakowski Medical Research Institute, PAS, Warsaw, Poland)

10.45 – 11.15 **Coffee Break**

11.15 – 13.15 **Symposium 13** [Hall 10 A/C]
Plasticity and encoding at synapses and neuronal networks and beyond
Chair: **Jerzy Mozrzymas** (Medical University, Wrocław, Poland)
Speakers: **Sebastiano Curreli**, **Andrea Barberis**, **Grzegorz Wiera**

Symposium 14 [Hall 10 B]
Bridging Autoimmunity and Neurodegeneration: Immune Cells in Action
Chair: **Natalia Małek** (Wrocław University of Science and Technology, Wrocław, Poland)
Speakers: **Bart Eggen**, **Maarten Titulaer**, **Marta Kamińska**, **Agnieszka Zabłocka**

Symposium 15 [Hall 10 D]
Time for flies – an alternative model for research on brain diseases
Chair: **Milena Damulewicz** (Jagiellonian University, Cracow, Poland)
Speakers: **Milena Damulewicz**, **Aron Szabo**, **Aaron Voigt**, **Sergio Casas-Tinto**

13.15 – 13.45 **Lunch Break**

13.45 – 15.00 **Poster session III**

15.00 – 16.00 **Keynote lecture** [Hall 10 A/C]
Drosophila as a model for human neurodegenerative disease: A focus on the brain through age & disease
Chair: **Elżbieta Pyza** (Jagiellonian University, Cracow, Poland)
Speaker: **Nancy Bonini** (University of Pennsylvania, USA)

16.00 – 16.30 **Official closing & Best Presentation Awards** [Hall 10 A/C]

PLENARY LECTURES

PL1. MOLECULAR BIOLOGY OF SYNAPTIC PLASTICITY

Leszek Kaczmarek
BRAINCITY, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Almost 40 years ago we have identified c-Fos, a component of AP-1 transcription factor, as playing a role in learning and memory, i.e., healthy mind. Next, we have identified such c-Fos/AP-1 gene targets in activated neurons as those encoding tissue inhibitor of metalloproteinases-1 (TIMP-1) and matrix metalloproteinase 9 (MMP-9). MMP-9 is an extracellularly operating enzyme that we and others have demonstrated to be an important regulatory molecule in control of synaptic plasticity, learning and memory. We have shown that either genetic or pharmacological inhibition of MMP-9 impairs long-term potentiation at various pathways, as well as appetitive and spatial memory formation, although aversive learning remains apparently intact in MMP-9 KO mice. MMP-9 is locally translated and released from the excitatory synapses in response to neuronal activity. Extrasynaptic MMP-9 is required for growth and maturation of the dendritic spines to accumulate and immobilize AMPA receptors, making the excitatory synapses more efficacious. Our studies on animal models have implicated MMP-9 in such neuropsychiatric conditions, as e.g., epileptogenesis, autism spectrum disorders, development of addiction, schizophrenia and depression. We have also reported that in humans MMP-9 appears to contribute to epilepsy, alcohol addiction, Fragile X Syndrome, schizophrenia and bipolar disorder. In aggregate, all those conditions may be considered as relying on alterations of dendritic spines/excitatory synapses and thus understanding the role played by MMP-9 in the synaptic plasticity may allow to elucidate the underpinnings of major neuropsychiatric disorders, i.e., diseased mind.

PL2. GUT FEELINGS – MICROBIOME, BRAIN AND BEHAVIOUR ACROSS THE LIFESPAN

John F. Cryan
University of Cork, Cork, Ireland

The microbiota-gut-brain axis is emerging as a research area of increasing interest for thoseinvestigating the biological and physiological basis of neurodevelopmental, age-related and neuropsychiatric disorders. The routes of communication between the gut and brain include the vagus nerve, the immune system, tryptophan metabolism, via the enteric nervous system or via microbial metabolites such as short chain fatty acids. Studies in animal models have been key in delineating that neurodevelopment and the programming of an appropriate stress response is dependent on the microbiota. Developmentally, a variety of factors can impact the microbiota in early life including mode of birth delivery, antibiotic exposure, mode of nutritional provision, infection, stress as well as host genetics. Stress can significantly impact the microbiota-gut-brain axis at all stages across the lifespan. Moreover, animal models have been key in linking the regulation of fundamental brain processes ranging from adult hippocampal neurogenesis to myelination to microglia activation by the microbiome. Finally, studies examining the translation of these effects from animals to humans are currently ongoing. Further studies will focus on understanding the mechanisms underlying such brain effects and developing nutritional and microbial-based psychobiotic intervention strategies and how these interact with various systems in the body across the lifespan.

PL3. A NEUROSCIENTISTS' JOURNEY INTO ENVIRONMENTAL NEUROSCIENCE. WHAT LARGE-SCALE SCALE HUMAN NEUROIMAGING CAN TELL US ABOUT THE IMPACT OF ENVIRONMENT AND SOCIETY ON BRAIN AND BEHAVIOR

Marcin Szwed

Institute of Psychology, Jagiellonian University, Cracow, Poland

The last decade has seen an expansion of large-scale MRI neuroimaging, with study populations reaching thousands of subjects. This expansion has made it possible, for example, to investigate the brain basis of individual psychological differences in personality and cognition. It has also enabled the growth of environmental neuroscience, which studies how environmental exposures such as air pollution influence brain function. Here I will discuss the results of the NeuroSmog study, which assesses how exposure to air pollution affects the developing brains of 741 schoolchildren in Southern Poland, a region characterized by very high levels of particulate air pollution. I will present diffusion, resting-state, and task fMRI results showing that both early-life and recent air pollution exposure significantly affect the brain, in particular neural circuits for attention and cognition. These results are in line with a large body of evidence showing associations between air pollution and increased ADHD incidence.

PL4. TIME AND PUNISHMENT: NEURAL CIRCUITS SHAPING THE ENCODING OF ADVERSITY

Andrew Holmes

National Institute on Alcohol Abuse and Alcoholism, NIH, USA

Adverse events act as powerful influences on our current and – via their encoding in memory – future behavior. Recent research has shone new light on the brain circuity that underpins how we learn, remember and adapt to adversity. Of note in this context are findings identifying complex and highly dynamic mechanisms, at the level of neural systems and neuronal populations, within the amygdala and its functionally interconnected cortical partners. This rapidly advancing field of basic research holds promise for elucidating a neurobiological basis for stress-related neuropsychiatry illness and its treatment.

PL5. OSCILLATORY BRAIN ACTIVITY AND THE DEPLOYMENT OF SELECTIVE ATTENTION

John J. Foxe

University of Rochester, Rochester, NY, USA

Both animal intracranial recordings and human scalp electrophysiological recordings make clear that neural oscillatory mechanisms play a critical role in sensory-perceptual and cognitive functions, including selective attention, working memory, and feature binding, to name a few. A variety of cognitive effects that are associated with specific brain oscillations have been reported, which range in spectral, temporal, and spatial characteristics depending on the context. A major focus of our group's work has been on investigating the role of alpha-band oscillatory activity (8-14 Hz) as a potential attentional suppression mechanism. Our work has shown that 1) phasic increases in alpha-band power are associated with suppression of visual inputs when individuals need to selectively attend to auditory inputs (i.e. cross-sensory suppression), 2) that topographically/retinotopically specific increases in alpha-power are associated with suppressing irrelevant visual inputs from specific parts of space when other parts of space contain the information to be acted upon (i.e. visuo-spatial suppression), and 3) that increases in alpha power within a given visual processing stream (i.e. dorsal versus ventral) results in feature-specific attentional deployments (i.e. feature-based suppression). In this presentation, we will discuss the evidence for a prominent role in attentional suppression for alpha-band oscillatory activity, and present evidence for deficits in this ability in certain clinical populations (e.g. Autism Spectrum Disorder) and enhancements of it in other populations (e.g. Deafness).

PL6. NEW INSIGHTS INTO THE BRAIN CONTROL OF FOOD CHOICE AND OBESITY

Lora Heisler

University of Aberdeen, Aberdeen, UK

Obesity has become one of the key medical and economic challenges. Glucagon-likepeptide-1 receptor (GLP-1R) agonists such as semaglutide (Ozempic) improve obesity by reducing food intake by acting a brain GLP-1Rs. Specifically how the reduction in food ingestion is achieved is still under investigation. Some reports indicate that semaglutide promotes changes in food preferences[1], though there are challenges to study this in the real world in people over time[2]. In a controlled laboratory setting, we investigated the effect of GLP-1R agonists on food and drink choice and preference in wild type dietary-induced obese mice. Our findings provide insight into one of the ways that GLP-1R agonists reduce caloric intake to promote the therapeutic effect of weight loss. References: [1] Blundell J, et al. Effects of once-weekly semaglutide on appetite, energy intake, control of eating, food preference and body weight in subjects with obesity. Diabetes Obes Metab., 2017. doi: 10.1111/dom.12932 [2] Bettadapura S, et al. Changes in food preferences and ingestive behaviors after glucagon-like peptide-1 analog treatment: techniques and opportunities. Int J Obes., 2024. doi: 10.1038/s41366-024-01500-y

PL7. DROSOPHILA AS A MODEL FOR HUMAN NEURODEGENERATIVE DISEASE: A FOCUS ON THE BRAIN THROUGH AGE & DISEASE

Nancy Bonini

University of Pennsylvania, USA

Our laboratory uses Drosophila melanogaster to study human neurodegenerative disease, and the risk factors. As part of these studies, we developed a head-specific traumatic brain injury (TBI) model. In this model, we found that an AP1 pathway became active acutely to protect from TBI, but then remained chronically elevated. Detailed analysis of the cells with chronic activation indicated that they have the properties of senescent cells. Study of these cells indicated they appear in response to mitochondrial decline and, if the pathway is dampened, they prolong health and lifespan. We are now using this system to perform genetic screens to discover new triggers of senescence, and how to protect the brain.

SYMPOSIA LECTURES

S1.1. ESTABLISHING UNIFIED, AUTOMATIC, UNBIASED PLATFORM FOR CHARACTERIZATION OF SOCIAL BEHAVIOR IN MICE

Bartosz Zglinicki

Polish Centre for Technology Development, Łukasiewicz – PORT, Wrocław, Poland

Validation of any biological intervention developed for tackling the root or symptoms of neuropsychiatric disorders requires proper tools. Disturbances of social behavior is a hallmark of many psychiatric conditions, such as depression, anxiety and schizophrenia. Capturing and quantifying broad range of social behavior simultaneously within a single tool is therefore essential, as it would allow for registration and clustering of groups of behavior specific for particular disorders. Such method would be of great advantage for better translational studies. Here, within Same-NeuroID project, we implemented a pipeline for efficient characterization of complex social behavior in mice. In the setup animals are housed together in a semi naturalistic environment with proper bedding, food and water access, and with night-day cycle. Animals are recorded for long hours to ensure a capture of diverse behavior. SLEAP.ai is used for pose estimation of recorded animals and extracted coordinates are processed by deepOF software to create set of features. These features are then used to "feed" models of both supervised and unsupervised learning for complex behavior classification.

FINANCIAL SUPPORT: Funding: This work was carrying out by the Horizon Europe Research and innovation funding programme under Grant Agreement 101079181 – SAME – NeuroID and the national science centre based on decision no. Dec-2021/41/b/nz3/04099 entitled „do astrocytes control synaptic connections in neural networks relevant to psychiatric diseases?". Grant agreement: umo-2021/41/b/nz3/04099 – AstroSyCo

S1.2. THE CEREBELLUM AS A DRIVER OF CORTICAL MATURATION AND COGNITIVE FLEXIBILITY

Aleksandra Badura

Erasmus Medical Center, Rotterdam, Netherlands

Understanding how the brain develops and how deviations from typical neurodevelopment are linked to health and disease remains a top priority in clinical neuroscience. Research to date has disproportionately focused on the development of the cerebrum, thereby omitting the so-called 'small brain', the cerebellum. While the cerebellum's involvement in motor control is well documented, recent studies have made clear that it also plays a crucial role in higher cognitive function and that disrupted cerebellar development distorts cortical maturation. However, little is known about mechanisms underlying this complex cerebellar-cortical interplay during development. Here we have addressed this knowledge gap by studying the influence of disrupted cerebellar development on cortical maturation and behavioural phenotypes. Specifically, we examined the effects of disrupted crus 1 development in mice, using targeted cerebellar ablations at distinct developmental stages to elucidate the timing-dependent nature of behavioral and anatomical outcomes. Our findings reveal that lesions in crus I result in impairments in social and flexible behaviors without affecting motor skills, with the phenotypic impact varying by the timing of the lesion. Further, using an ultra-high field 7T MR imaging we have investigated anatomical differences across experimental groups. Through deformation-based morphometry, images were processed and analysed at the voxel level. Volume changes at the voxel level were then used to accurately retrieve changes in structure volumes through atlas registration. This approach allowed for patterns of deformation to be linked to patterns in behavioural defects at voxel and structural levels, as well as between individuals and experimental groups. Together, our results elucidate a developmental stage-specific effects of early cerebellar injury on behavioral phenotype and whole-brain anatomy. Such insights highlight the existence of critical periods that influence the cerebello-cortical development, potentially providing predictive value for neurodevelopmental deficits' severity following cerebellar disruption.

S1.3. CAGE OF THRONES: AUTOMATED ANALYSIS OF SOCIAL BEHAVIOUR AND POWER DYNAMICS IN MOUSE HIERARCHIES

Adam Brosnan

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Social hierarchies are thought to regulate social structures. In humans, social hierarchies manifest based on factors such as wealth, knowledge, age, and strength, which determine resource allocation. For example, in capitalist societies, wealth determines how much of a resource a person is able to obtain. In wild mice, a dominant mouse often has more access to territory, food, and mates. In this experiment, mouse social dominance is determined by chasing behaviour in a fully automated, semi-naturalistic, home cage monitoring system called 'Eco HAB.' Using this apparatus, we aim to model social dominance in socially housed (n=30) C57BL/6 mice. We focus on three questions: (1) How quickly do mice form social hierarchies in Eco-HAB? (2) Once formed, are they stable? (3) Are they flexibly reformed across different social contexts? The automation of data collection and analysis in Eco-HAB offers significant advantages in studying social dynamics. By enabling continuous, non-invasive monitoring of behavior, the system provides high-resolution data that reveal intricate patterns of dominance and territoriality. Results demonstrate that mice form a hierarchy within 2 to 3 days, which, once established, is stable. In contexts exclusively composed of dominant animals, instead of regulating their society via chasing behaviour, animals switch to territoriality. The automated analysis streamlines the identification of these behaviors, reduces observer bias, and allows for the scalable study of multiple groups, making it a powerful tool for uncovering the complexities of social dominance.

S1.4. THE BEHAVIORAL LANGUAGE OF STRESS AND TREATMENT RESPONSE: FROM COMPLEX SOCIAL BEHAVIORS TO MOLECULAR CIRCUITS

Juan Pablo Lopez

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Dr. Juan Pablo Lopez is an Assistant Professor in the Department of Neuroscience at Karolinska Institutet. His research focuses on decoding the behavioral language, molecular mechanisms, and cellular circuits underlying stress-related psychiatric disorders and their treatments. His lab addresses key questions in translational psychiatry, such as: Why do some individuals develop psychiatric symptoms after trauma while others remain resilient? When during development does adversity become biologically embedded? What molecular changes accompany clinical improvement, and what are the biological correlates of treatment response? To explore these questions, Dr. Lopez's team employs a multidisciplinary approach that integrates state-of-the-art molecular, cellular, and computational neuroscience tools. Their goal is to bridge the gap between preclinical models and human psychiatry. In this talk, he will present recent findings on cell-type-specific molecular mechanisms involved in responses to acute and chronic stress, as well as the rapid and sustained antidepressant effects of novel treatments such as ketamine and psychedelic compounds. He will also highlight the use of automated behavioral tracking systems to analyze complex social behaviors in group-housed mice, showcasing how unbiased behavioral analysis can reveal new insights into stress and treatment response.

S2.2. IMPACT OF ZINC (DYS)HOMEOSTASIS ON THE DEVELOPMENT OF THE AXON AND ITS INITIAL SEGMENT

Jerome Ezan

Neurocentre Magendie – Inserm U1215, Bordeaux University, France

Exploring the role of Zinc homeostasis during the formation of the axon intial segment (AIS). Many neurodevelopmental disorders (NDDs), including autism spectrum disorders (ASDs), involve genetic and environmental factors like zinc deficiency, with comorbidities such as epilepsy. While Zinc's role in synaptic transmission is well-studied[1], its impact on the Axonal Initial Segment (AIS), the site of electrical signal transmission, remains unclear. We recently identified the Planar Cell Polarity pathway (PCP) protein Prickle

2 (Pk2) as a key determinant of neuronal polarity, essential for AIS assembly and axonal excitability[2]. By investigating the signaling pathways/kinases involved downstream of Zinc in neuronal polarization and AIS development, our study explores the hypothesis that Zinc and the PCP pathway may converge during this process. References: [1] Błażewicz, A, Grabrucker A. M. Metal Profiles in Autism Spectrum Disorders: A Crosstalk between Toxic and Essential Metals. Int. J. Mol. Sci. 24, 308, 2022. [2] Dorrego-Rivas A, et al. The core PCP protein Prickle2 regulates axon number and AIS maturation by binding to AnkG and modulating microtubule bundling. Sci. Adv. 8, eabo6333, 2022.

S2.3. ZINC AS A REGULATOR OF NEUROINFLAMMATORY SIGNALLING IN AUTISM SPECTRUM DISORDERS

Andreas M. Grabrucker

University of Limerick, Bernal Institute, Limerick, Ireland

An impaired development of neural circuitry has been proposed as key pathology of Autism Spectrum Disorders (ASD). Astrocytes are important regulators of neuronal development and activity. Increased reactive astrocytes were reported in ASD and may significantly impact the balance between synapsę maturation and elimination, thereby modulating neural circuitry. Therefore, attenuating astrocyte activation may be an important approach for preventing and treating ASD. Intriguingly, zinc deficiency has been consistently linked to increased pro-inflammatory signalling and ASD[1]. We identified a cellular zinc-dependent signalling pathway that leads to astrocyte activation[2]. In this talk, the mechanism of how low zinc levels activate inflammatory-driven crosstalk between astrocytes and neurons is presented. References: [1] Sauer AK, et al. Prenatal Zinc Deficient Mice as a Model for Autism Spectrum Disorders. Int J Mol Sci., 2022. doi: 10.3390/ijms23116082 [2] Stanton J, et al. Zinc signaling controls astrocyte-dependent synapse modulation via the PAF receptor pathway, J Neurochem., 2024. doi: 10.1111/jnc.16252

S2.4. ZINC DEFICIENCY AND CHRONIC STRESS: EXPLORING THEIR IMPACT ON DEPRESSION AND ANTIDEPRESSANT EFFECTIVENESS

Bernadeta Szewczyk

Maj Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

Clinical and preclinical studies provide evidence that chronic stress and nutritional deficits, particularly in dietary zinc (Zn) intake, may act as risk factors for the development of major depressive disorder (MDD). Additionally, there may be potential links between low serum Zn levels and the emergence of treatment-resistant depression. This talk will explore the effects of chronic restraint stress (CRS) and a low-zinc diet (ZnD) on the efficacy of antidepressants in mice. Furthermore, the underlying mechanisms responsible for these effects will be discussed. References: 1. Pochwat B, et al. Combined hyperforin and lanicemine treatment instead of ketamine or imipramine restores behavioral deficits induced by chronic restraint stress and dietary zinc restriction in mice. Front Pharmacol., 2022, doi: 10.3389/fphar.2022.933364

S3.1. SOMATOSENSATION AND CANCER NEUROSCIENCE

Mateusz Kucharczyk

Polish Centre for Technology Development, Łukasiewicz – PORT, Wrocław, Poland

Dr. Kucharczyk will explore the neurogenic regulation of cancer and associated pain, focusing on the role of peptidergic and silent nociceptors in cancer-induced bone pain. Using selective genetic targeting, he will showcase how these neuronal populations respond to pathological changes in cancerous tissue. Finally, he will present novel approaches for studying presynaptic modulation with fibre-type-specific functional imaging, highlighting the modulation potential of nociceptive transmission through genetically-defined spinal and descending pathways. References: 1. Kucharczyk MW, et al. The impact of bone cancer on the peripheral encoding of mechanical pressure stimuli. Pain, 2020. doi: 10.1097/j.pain.0000000000001880 2. Kucharczyk MW, et al. A critical brainstem relay for mediation of diffuse noxious inhibitory controls. Brain. 2023. doi: 10.1093/brain/awad002

S3.2. TARGETED CHEMOGENETIC INHIBITION OF SENSORY AFFERENTS FOR PAIN RELIEF IN MICE AND HUMANS

Jimena Perez-Sanchez

Neural Injury Group, University of Oxford, UK

Dr. Perez-Sanchez will discuss her work with the PSAM4-GlyR chemogenetic system. This system uses humanized ligand-gated channels to achieve selective silencing of hyperexcitable neurons with demonstratable therapeutic potential across multiple pain conditions, including inflammatory joint and neuropathic. Her findings reveal how chemogenetic approaches can precisely control specific neuronal compartments, extending their application to study presynaptic control mechanisms and the role of resting potential differences in DRG neurons. References: 1. Perez-Sanchez J, et al. A humanized chemogenetic system inhibits murine pain-related behavior and hyperactivity in human sensory neurons. Science Translational Medicine, 2023. doi: 10.1126/scitranslmed.adh3839

S3.3. ASSESSMENT OF SPONTANEOUS ACTIVITY AND THE ROLE OF SILENT NOCICEPTORS IN MUSCULOSKELETAL PAIN

George Goodwin

Wolfson Sensory, Pain, and Regeneration Centre, King's College London, UK

Dr. Goodwin will discuss methods for assessing spontaneous activity in dorsal root ganglion neurons, as well as the use of a selective genetic model that enable targeted studies of silent nociceptors. His research provides new insights into how spontaneous neuronal activity underpins chronic pain states, particularly in musculoskeletal conditions with neuropathic features, such as osteoarthritis and rheumatoid arthritis. References: 1. Ingram S, et al. Assessing spontaneous sensory neuron activity using in vivo calcium imaging. Pain, 2024. doi: 10.1097/j.pain.0000000000003116 2. Choi D, et al. Spontaneous activity in peripheral sensory nerves: a systematic review. Pain, 2024. doi: 10.1097/j.pain.0000000000003115

S3.4. USING 3D IMAGING AND SPATIAL BRAIN TRANSCRIPTOMICS TO ANALYZE PERIPHERAL RELIEF OF NEUROPATHIC PAIN

Sara Jager

Madsen Lab, Department of Neuroscience, University of Copenhagen, Denmark

Dr. Jager will focus on the application of tissue clearing and 3D imaging techniques to investigate peripheral tissues and their interactions with the nervous system during neuropathic pain. Additionally, she will highlight the integration of whole-brain cFos analysis to map the neural response in the brain to manipulation of peripheral inputs. Dr. Jager will further demonstrate how spatial brain transcriptomics enhances these analyses, offering deeper insights into the molecular underpinnings of pain processing and relief. References: 1. Jensen KL, et al. Peripherally restricted PICK1 inhibitor mPD5 ameliorates pain behaviors in murine inflammatory and neuropathic pain models. JCI Insight., 2024. doi: 10.1172/jci.insight.170976

S4.1. SEX-SPECIFIC CONSEQUENCES OF EARLY LIFE ADVERSITY: FROM TRANSCRIPTOME TO COMPLEX BEHAVIOR

Mathias Schmidt

MPI Munich, Munich, Germany

Early-life stress (ELS) can profoundly shape adult physiology and behavior, leading to resilience or vulnerability to psychopathology. Using rodent models, we employ deep phenotyping methods, such as machine-learning assisted behavioral monitoring with DeepOF, to identify enduring behavioral consequences of ELS that significantly impact the animals' fitness and health. These behaviors, including complex social behaviors, are linked to alterations in neuronal activation, physiology, transcriptome, and metabolome. Notably, we observe pronounced sex differences in both the behavioral and physiological outcomes of ELS, as

well as in the underlying molecular mechanisms. Our findings underscore the importance of developing sex-specific treatment interventions to enhance individual stress resilience and provide effective therapeutic approaches. References: 1. Brix LM, et al. Metabolic effects of early life stress and pre-pregnancy obesity are long lasting and sex specific in mice. Eur J Neurosci., 2023. doi: 10.1111/ejn.16047

S4.2. LONG-TERM EFFECTS OF EARLY-LIFE STRESS ON COGNITION AND EMOTIONAL FUNCTIONS: A SYNERGISTIC ACTION OF STRESS, INFLAMMATION AND NUTRITION

Aniko Korosi

University of Amsterdam, Amsterdam, Netherlands

Early-life stress is associated with increased vulnerability to cognitive impairments later in life. We investigate the role of a synergistic effect of stress, metabolic factors, nutrition and the neuroimmune system in this early-life induced programming. Using a model where mice face limited nesting material during first post-natal week, we analyze long-term brain effects under both normal and challenging conditions (e.g., LPS, amyloid in Alzheimer's models, and exercise). We also explore cognitive, emotional functions, neurogenesis, and microglial responses, focusing on essential nutrients like fatty acids and polyphenols as protective interventions. Our findings reveal an impairment of hippocampal neurogenesis and priming of microglia for exaggerated inflammatory responses by ELS. Early dietary intervention can partly mitigate these effects, offering insights for targeted nutrition in vulnerable populations. References: 1. Reemst K, et al. Molecular underpinnings of programming by early-life stress and the protective effects of early dietary ω6/ω3 ratio, basally and in response to LPS: Integrated mRNA-miRNAs approach. Brain Behav Immun., 2024. doi: 10.1016/j.bbi.2024.01.011 2. Kotah JM, et al. Early-life stress and amyloidosis in mice share pathogenic pathways involving synaptic mitochondria and lipid metabolism. Alzheimers Dement., 2024. doi: 10.1002/alz.13569

S4.3. TOWARDS THE BIOLOGICAL PLAUSIBILITY AND BIOMARKERS OF INTERGENERATIONAL TRAUMA IN HUMANS

Ali Jawaid

Polish Centre for Technology Development, Łukasiewicz – PORT, Wrocław, Poland

Childhood trauma is a major risk factor for adult psychiatric and physical disorders, with recent research indicating these effects may extend across generations. We investigate the molecular basis of the long-term effects and intergenerational transmission of trauma. We examine small non-coding RNAs in serum, sperm, and milk from diverse trauma-exposed cohorts, including Pakistani children and men with histories of complex childhood trauma, Polish mothers with exposure to adverse childhood experiences, and Bosnian adults who were exposed to the genocide during their childhood. Using a parallel mouse model of post-natal trauma involving unpredictable maternal separation and stress, our findings suggest that lipid related circulating microRNAs play a critical role in trauma transmission across generations, highlighting their potential as biomarkers and providing insights into therapeutic strategies. References: 1. Tomszewska W, et al. Differential microRNAs and metabolites in the breast milk of mothers with adverse childhood experiences correlate with offspring temperament. (In review: Translational Psychiatry) 2. Jawaid A, et al. miRNA differentially expressed in human serum and sperm after childhood trauma potentially impact offspring health. (In revision: Biological Psychiatry)

S4.4. INTERPLAY OF SERUM LIPIDS AND MICROGLIA IN THE SUSCEPTIBILITY TO THE LONG-TERM BEHAVIORAL EFFECTS OF ADVERSE CHILDHOOD EXPERIENCES

Weronika Tomaszewska[1,2]

[1] *Nencki Institute of Experimental Biology*

[2] *Łukasiewicz – PORT, Wrocław, Poland*

Adverse childhood experiences (ACE) significantly increase the risk of adult-onset neuropsychiatric disorders. However, the mechanisms underlying susceptibility versus resilience to the long-term effects of ACE remain largely unknown. We hypothesize that microglia centrally integrate lipid-mediated signals and alter their inflammatory and phagocytic outputs to determine the susceptibility *vs.* resilience to ACE. Synergizing in vitro analyses of microglia incubated with samples from human ACE cohorts in Pakistan and Bosnia, we demonstrate that microglial metabolism and phagocytosis of healthy synapses are differentially regulated upon stimulation with serum from resilient *vs.* susceptible individuals. Our ongoing investigations focus on dissecting the individual contribution of peripheral lipids and their associated non-coding RNAs in alteration of microglial functions to determine susceptibility *vs.* resilience to ACE. References: 1. https://www.neuron-eranet.eu/projects/MUSE+ACE/

S5.2. PRODROMAL MODEL OF PARKINSON'S DISEASE BASED ON TARGETING NORADRENERGIC SYSTEM

Grzegorz Kreiner

Department of Brain Biochemistry, Maj Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland

Parkinson's disease (PD) is characterized by progressive loss of dopaminergic neurons of substantia nigra (SN) and ventral tegmental area (VTA), directly responsible for symptomatology. However, PD is associated with malfunctions in the noradrenergic system as well. Degeneration of locus ceruleus (LC) in PD patients may even exacerbate the loss of SN/VTA neurons. Recently, we have shown that enhancement of noradrenergic transmission can have beneficial effects in transgenic mouse model of progressive parkinsonism. On the other hand, our newly created mouse model of selective degeneration of LC influenced negatively functioning of the dopaminergic system. These mice may become a valuable tool to study the prodromal phase of PD and neuroprotective therapies. References: 1. Braak et al. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging., 2003. doi: 10.1016/s0197-4580(02)00065-9 2. Barut et al. Genetic lesions of the noradrenergic system trigger induction of oxidative stress and inflammation in the ventral midbrain. Neurochem Int., 2022. doi: 10.1016/j.neuint.2022.105302

S5.3. RELEVANCE OF ISOLATED REM SLEEP BEHAVIOUR DISORDER AS PRODROMAL SYNUCLEINOPATHY

Ambra Stefani

Medical University of Innsbruck, Innsbruck, Austria

Isolated REM sleep behaviour disorder (iRBD) represents an early stage synucleinopathy. Clinical aspects will be presented, as well as challenges in diagnosis and screening with insights into new methods to overcome them. The speaker will also provide an overview of biomarkers in iRBD, addressing different types of biomarkers and their potential role in the context of neuroprotective/neuromodulating trials. References: 1. Miglis MG, et al. Biomarkers of conversion to α-synucleinopathy in isolated rapid-eye-movement sleep behaviour disorder. Lancet Neurol., 2021. doi: 10.1016/S1474 4422(21)00176-9 2. Högl B, et al. Rapid eye movement sleep behaviour disorder: Past, present, and future. J Sleep Res., 2022. doi: 10.1111/jsr.13612

S5.4. SALIVARY BIOMARKERS IN PRODROMAL SYNUCLEINOPATHIES AND PARKINSON'S DISEASE

Giorgio Vivacqua
Campus Biomedico University of Roma, Rome, Italy

A mismatch between clinical and neuropathological onset of synucleinopathies hampers the efficacy of disease-modifying therapies, calling for pre-clinical molecular diagnosis. Salivary glands are richly innervated by visceral fibres which enter in strict contact with secretory adenomeres, making saliva a source of biomarkers for neurological disorders. Altered salivary biomarkers were found in saliva of patients affected by different synucleinopathies. RT-QuIC assay reveals seeding-competent alpha-synuclein in PD patients saliva and correlates with disease severity. Alterations in autophagic and inflammatory markers enable molecular clustering of PD patients and are observed in iRBD subjects. This makes saliva a key biofluid candidate for diagnosis of prodromal synucleinoapthies and for early discrimination of different clinic-molecular subtypes of PD. References: 1. De Bartolo MI, et al. A Combined Panel of Salivary Biomarkers in de novo Parkinson's Disease. Ann Neurol., 2023. doi: 10.1002/ana.26550 2. Vivacqua G, et al. Salivary α-Synuclein RT-QuIC Correlates with Disease Severity in de novo Parkinson's Disease. Mov Disord., 2023. doi: 10.1002/mds.29246

S6.1. COPPER TOXICITY IN THE BRAIN – EXPOSURE, MECHANISMS AND MANIFESTATIONS

Susan Gaskin
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Copper toxicity disrupts normal brain function through catecholamine imbalance, abnormal myelination of neurons and loss of normal brain architecture, manifesting in a wide spectrum of neurologic and/or psychiatric symptoms[1]. In the brain, copper is tightly regulated to prevent oxidative stress, which decreases neuronal viability by disrupting signalling pathways regulating the regeneration, survival and repair of neurons. Increased copper enters the brain via the endothelial cell layer of the blood-brain-barrier, which is vulnerable to impaired mitochondrial respiration from cuprotosis, resulting in leakiness even at modest circulating labile copper levels[2]. The latter can result from environmental exposure via ingestion of copper salts, e.g. pesticide residues, absorbed directly into the blood stream. References: [1] Lutsenko S, et al. Copper and the brain noradrenergic system. J Biol Inorg Chem., 2019. doi: 10.1007/s00775-019-01737-3. [2] Borchard S, et al. The exceptional sensitivity of brain mitochondria to copper. Toxicology in vitro, 2018 doi: 10.1016/j.tiv.2018.04.012

S6.2. WILSON DISEASE – GENETICS, CLINIC, DIAGNOSIS AND TREATMENT

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Wilson Disease is a rare autosomal recessive disorder (30-50 cases per million) caused by mutation(s) in the ATP7B gene. Impaired function of the ATPase in liver cells leads to impaired incorporation of copper onto ceruloplasmin and its excretion in the bile, leading to accumulation primarily in the liver and the brain. Clinical symptoms are highly variable in age of onset, dominant organ symptoms and their intensity. Diagnosis is based on clinical signs (hepatic, neurological, psychiatric), measurement of serum ceruloplasmin, urine copper excretion and mutation analysis of ATP7B. Pre-symptomatic diagnosis is possible. Copper depleting treatment (chelation or inhibition of copper absorption) is successful, but must be lifelong. Gene therapy is under development. References: 1. Roberts EA, et al. Current and Emerging Issues in Wilson's Disease. N Eng J Med., 2023. doi: 10.1056/NEJMra1903585 2. Czlonkowska A, et al. Seven decades of clinical experience with Wilson's disease: Report from the national reference centre in Poland. Eur J Neurol, 2022. doi: 10.1111/ene.15646

S6.3. COPPER AND OTHER NEUROLOGICAL DISORDERS

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Copper, a trace element, is an essential a cofactor for many cuproenzymes involved in neuronal development, myelination, neurotransmitter synthesis, oxidative metabolism and antioxidant defense (e.g. cytochrome C oxidase, superoxide dismutase and dopamine-beta hydroxylase)[1]. Wilson's disease, the best-known disorder of copper metabolism, has pathological copper accumulation in different organ systems[2]. Other less frequent neurodegenerative disorders with copper metabolism disturbances, falling within the broad range of Wilson's disease phenotypes, include Menkes disease, Mednik disease, apoceruloplasminemia (with copper and iron metabolism disturbances), Huppke-Brendel syndrome, MDR3 deficiency, manganese transport defects and congenital glycosylation disorders[1,2]. These syndromes document how aspects of copper metabolism may be altered, other than the defect in ATP7B of Wilson's disease[2]. References: [1] Gale J, et al. The physiological and pathophysiological roles of copper in the nervous system. Eur J Neurosci., 2024. doi: 10.1111/ejn.16370 [2] Bandmann O, et al. Wilson's disease and other neurological copper disorders. Lancet Neurology, 2015. doi: 10.1016/S1474-4422(14)70190-5

S6.4. NEUROIMAGING OF COPPER DISORDERS

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Neuroimaging techniques, including MRI and PET scans, are valuable tools for studying brain changes in disorders associated with abnormal copper metabolism, such as Wilson's disease. Neuropathology in these disorders often includes demyelination, gliosis, basal ganglia lesions, white matter abnormalities, and atrophy, all of which can be detected on structural MRI[1]. Concomitant abnormalities of other metals are common. Advanced techniques like diffusion tensor imaging (DTI) and spectroscopy further reveal microstructural damage and metabolic disruptions. PET imaging, using radiolabeled copper isotopes such as 64Cu, allows researchers to visualize copper uptake, distribution, and clearance in the brain over time[2]. Neuroimaging is instrumental in understanding the pathophysiology of copper disorders, monitoring disease progression, and assessing the efficacy of treatments. References: [1] Dusek P, et al. Semiquantitative Scale for Assessing Brain MRI Abnormalities in Wilson Disease: A Validation Study. Mov Disord., 2020. doi: 10.1002/mds.28018 [2] Munk DE, et al. Distribution of non-ceruloplasmin-bound copper after i.v. 64Cu injection studied with PET/CT in patients with Wilson disease. JHEP Rep., 2023. doi: 10.1016/j.jhepr.2023.100916

S7.1. 3D HUMAN ORGANOIDS TO EXPLORE THE DEVELOPMENT OF THE CEREBRAL CORTEX AND ITS BARRIER

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Cerebral cortex development is influenced by genetic, activity and environmental factors that shape neuronal diversity, synaptic connectivity, and network formation. Using cortical organoids, we model key developmental aspects, focusing on the emergence and modulation of spontaneous activity and circuit formation. This is relevant for understanding infantile epilepsy, where early activity patterns are altered. Combining multiomics and calcium imaging, we aim to uncover mechanisms underlying early epilepsies, to identify new therapies. We also investigate the role of the choroid plexus (ChP) and other barriers in supporting cortical maturation, activity, and network integration. To this end, we developed a ChP Org model that replicates the histological, functional, and ultrastructural features of native human ChP, able to respond to external stimuli.

S7.2. CORTICAL INTERNEURON MIGRATION IN THE FOREBRAIN – AN EVO-DEVO PERSPECTIVE

Antonela Bonafina

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The cerebral cortex contains excitatory projection neurons (PNs) and inhibitory interneurons (cINs). Most cINs originate in the ventral forebrain and migrate tangentially to integrate into cortical circuits. Prior research in mice showed that CCP1 (cytosolic carboxypeptidase 1) regulates cIN migration by deglutamylating myosin light chain kinase. Loss of CCP1 disrupts cIN migration, indirectly increasing PN generation and affecting cortical development. This study explores CCP1's role in human cIN migration using primary human forebrain cultures and cerebral assembloids. Disrupting CCP1 expression impairs migration, altering the motion from saltatory to gliding. While CCP1 similarly regulates nucleokinesis in human and mouse cINs, human cINs uniquely experience disrupted leading process branching, indicating an evolved CCP1 function in human cIN migration.

S7.3. HUMAN ASTROCYTES – EVOLUTION AND LINK TO NEURODEVELOPMENTAL DISEASES

Aleksandra Pękowska

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Astrocytes are the gatekeepers of brain homeostasis. Yet, apart from the housekeeping functions, astrocytes regulate synapse formation, activity, and pruning – processes that underlie higher-level brain functions and are critical for brain evolution. Using in-vivo data and human, chimpanzee, and macaque induced pluripotent stem cell-derived astrocytes (iAstrocytes), we recently uncovered that the expression of intellectual disability-related genes including CTCF, a factor orchestrating the three-dimensional chromatin structure, is progressively downregulated in the human astrocyte evolution. However, the relevance of this process to brain development and evolution remains unclear. I will present data illustrating broad changes in the CTCF-mediated chromatin topology in primate astrocytes and link these changes to the development of the human nervous system. Likewise, I will highlight the contribution of aberrant chromatin structure in astrocytes to neuronal development. References: 1. Ciuba K, et al. Molecular signature of primate astrocytes reveals pathways and regulatory changes contributing to the human brain evolution. bioRxiv, 2023. doi: 10.1016/j.stem.2024.12.011 2. Vian L, et al. The Energetics and Physiological Impact of Cohesin Extrusion. Cell, 2018. doi: 10.1016/j.cell.2018.03.072

S7.4. MODELLING NEURODEVELOPMENTAL PATHOGENESIS OF HUNTINGTON'S DISEASE WITH HUMAN IPSC LINES AND BRAIN ORGANOIDS

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Huntington's disease (HD) is a polyglutamine neurodegenerative disease involving neurodevelopmental pathogenesis. We developed a new model, fused dorso-ventral forebrain organoids mimicking the affected brain regions in HD and exhibiting significant growth and altered gene expression, suggesting that cells in HD brains represent specific phenotypes that favor increased proliferation over differentiation. In the HD models, we observed a strong increase in the cellular population of choroid plexus occurring in the blood-brain barrier (BBB). In addition, the upregulation of the choroid plexus marker TTR in mouse embryos and blood serum suggests its potential significance in HD pathogenesis. Our findings, may facilitate early detection and monitoring of HD and could enable the development of novel HD therapies targeting the choroid plexus. References: 1. Wiatr K, et al. Huntington Disease as a Neurodevelopmental Disorder and Early Signs of the Disease in Stem Cells. Mol Neurobiol., 2018. doi: 10.1007/s12035-017-0477-7 2. Świtońska-Kurkowska K, et al. Identification of neurodevelopmental organization of the cell populations of juvenile Huntington's disease using dorso-ventral HD organoids and HD mouse embryos. bioRxiv, 2024. doi: 10.1101/2024.09.23.614496

S8.1. EXPLORING BRAIN ACTIVITY MODULATED BY PSYCHOACTIVE SUBSTANCES IN RATS AND HUMANS

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Animal models play a critical role in elucidating complex neural processes, yet translating these findings into human studies remains challenging. Our research employs a cross-species approach to examine the effects of psychedelics on brain activity, focusing on functional connectivity and neural network dynamics. We analyze visual evoked potentials in rats to explore how sensory stimuli influence brain activity and identify event-related potential patterns associated with these neural responses. Further, we investigate the effect of psilocybin on resting-state EEG both in rats and humans. We utilize EEG source localization technique to identify the brain regions involved in these processes[1]. By integrating these diverse methodologies, we aim to bridge the gap between preclinical research and clinical applications. References: [1] Jiricek S, et al. Electrical Source Imaging in Freely Moving Rats: Evaluation of a 12-Electrode Cortical Electroencephalography System. Front Neuroinform., 2021. doi: 10.3389/fninf.2020.589228

S8.2. FLEXIBLE SILICONE IMPLANTS FOR STANDARDIZED RAT ECOG RECORDINGS

Theodor Doll

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For high-resolution data, electro-neurophysiology needs the lowest electrical impedances, high signal-to-noise ratios and high local resolution. While the latter could be realized by a high density of electrodes, the former requires highly flexible mechanical solutions. In both cases, the density of the electrical supply lines forms the bottleneck, so a lot can be achieved with elastic silicone on which conductive channels are printed, and for human applications the cortical implants can be made to fit snugly against the target tissue[1]. However, the rat experiments conducted as part of the RATCON project are pushing the technologists to the limits of what is feasible. New approaches are therefore required that go beyond the state of the art, e.g. hybrid and multiplexing approaches[2]. References: [1] Fütterer L, et al. Microdispenser 3D Printing. Transactions on Additive Manufacturing Meets Medicine, 2023. https://doi.org/10.18416/AMMM.2023.2309844 [2] Foremny K, et al. Biocompatibility Testing of Liquid Metal as an Interconnection Material for Flexible Implant Technology. Nanomaterials, 2021. doi: 10.3390/nano11123251

S8.3. MODELING OF RAT HEAD PHANTOMS FOR IMPLANT VALIDATION

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Since location of electrodes on the skull is considered in the newly developed implant, the effect of the skull on the measured EEG signals has to be evaluated. The forward EEG modeling plays a crucial role in solving source localisation and consequently in the detection of EEG connectivity networks in the rat brain.In this contribution we present the results of our investigation related to the influence of skull tissue electrical parameters, the spatial distribution of inhomogeneities and anisotropy, and the thickness of the skull on the measured potentials on the skull surface. The investigation is carried out on a simplified and realistic shape of a rat head phantom.

S8.4. EFFECTS OF GEOMETRY AND CONDUCTIVITY ON CURRENT SOURCE DENSITY ESTIMATION IN REALISTIC SCENARIOS

Marian Dovgialo

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Multielectrode EEG and intracranial recordings suffer from volume conductivity blurring out the signals in the spatial domain. To counter this we estimate current source density. Our kernel current source density distribution estimation method (kCSD1), unlike eLORETA, MNE or dSPM methods, is monopolar and well suited for high density implanted electrodes, where sources could be estimated on a cellular level and point dipole assumption might not hold true. KCSD can also be applied to estimate CSD in the whole brain and arbitrary meaningful regions. Here we discuss the effects of brain geometry and varying conductivity on the resulting solutions. We illustrate the results with examples of rat and human cases. References: 1. Chintaluri C, et al. What we can and what we cannot see with extracellular multielectrodes. PLoS Comput. Biol., 2021. https://doi.org/10.1371/journal.pcbi.1008615

S9.1. DIABETIC ENCEPHALOPATHY IN PRECLINICAL MODELS OF DIABETES MELLITUS: WHICH ROLE FOR NEUROACTIVE STEROIDS?

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Patients with diabetes mellitus (DM) often experience diabetic encephalopathy, leading to a higher risk of cognitive decline, dementia, and Alzheimer's disease, though the exact mechanisms remain still elusive[1]. Compelling data, obtained both in clinical settings and in animal models of DM, indicates that cognitive impairment may result from multiple contributing factors, including neuroinflammation, oxidative stress, mitochondrial dysfunction, and abnormal synaptic formation. This pathological state also disrupts neuroactive steroid levels in both plasma and the brain. These steroids are crucial regulators of neural function and have demonstrated neuroprotective effects in various neuropathological conditions[2]. This presentation will cover findings from preclinical models of DM on this subject. References: [1] Riederer P, et al. The diabetic brain and cognition. J. Neural. Transm., 2017. doi: 10.1007/s00702-017-1763-2 [2] Melcangi R.C., Giatti, S., Garcia-Segura, L.M. Levels and actions of neuroactive steroids in the nervous system under physiological and pathological conditions: Sex-specific features. Neurosci. Biobehav. Rev., 2016. doi: 10.1016/j.neubiorev.2015.09.023

S9.2. LACTOCRINE-BASED MECHANISMS RESPONSIBLE FOR REPROGRAMMING OF REPRODUCTIVE FITNESS OVER GENERATIONS

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Environmental factors shape the developmental trajectories of organisms and influence overall physiological well-being. Maternal diet deficits can significantly impact offspring health, including reproductive fitness. Our research explores lactocrine-based mechanisms that program reproductive fitness across generations by studying key signaling pathways, including the CNS, gonads, gamets, and peripheral factors, such as leptin. We found that transient undernutrition during the early postnatal period led to sex-specific neuroendocrine responses affecting reproduction in two generations. This not only influenced the timing of puberty but also altered the hormonal and molecular environments of the hypothalamic pituitary-gonadal axis, ultimately impacting reproductive efficacy in both males and females. Our findings emphasize the role of parental nutritional history in shaping the developmental paths of future generations, with early-life impacts. References: 1. https://pubmed.ncbi.nlm.nih.gov/27146259/ 2. Results of current project (publications under under review/in preparation – https://projekty.ncn.gov.pl/opisy/432953-en.pdf)

S9.3. HORMONAL MODULATION DURING DEVELOPMENT OF THE EFFECTS OF MALNUTRITION

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The early stages of life are crucial for the development of the neurohormonal systems that regulate feeding. Hormones such as leptin or ghrelin are involved in the programming of the hypothalamic feeding circuit. Studies carried out by our group have shown that the presence of gonadal steroids during the first postnatal weeks is also necessary both for the programming of the energy metabolism and feeding circuits and for the modulation of the alterations produced by the intake of high-fat or low-protein diets from the early stages of gestation and during development. In addition, these programming and modulating functions are sexually differentiated in males and females and depend on the hormone considered[1,2]. References: [1] Carrillo B, et al. Blocking of estradiol receptors ERα, ERβ and GPER during development differentially alters energy metabolism in male and female rats. Neuroscience., 2020. doi: 10.1016/j.neuroscience.2019.11.008 [2] Carrillo B, et al. Physiological and brain alterations produced by high fat diet in male and female rats can be modulated by increased levels of estradiol during critical periods of development. Nutr.Neurosci., 2019. doi: 10.1080/1028415X.2017.1349574

S9.4. HOW PRENATAL EXPOSURE TO CAFETERIA DIET, WHICH LEADS TO OBESITY AFFECT THE REPRODUCTION?

Joanna H. Śliwowska

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Environmental factors, such as diet, influence the development and health outcomes of offspring. The maternal overnutrition via access to cafeteria (CAF) diet in animals, which mimics the Western diet consumed by humans, can reprogram endocrine systems, leading to various diseases, including metabolic and reproductive ones. Our research explores mechanisms via which the CAF diet alters metabolic and reproductive outcomes, emphasizing sex differences. We have found that CAF material diet affects body weight and composition in offspring, alters metabolic profiles, and leads to inflammation and delayed puberty in female offspring. Moreover, we have found that the above changes are sex-specific and occur in the Kiss1/Gpr54 and Sirt1 systems in the brain and liver in the offspring. Thus, we have concluded that the mother's CAF diet leads to sex-specific alterations in metabolic and reproductive outcomes via the Kiss1/Gpr54 and Sirt1 systems in offspring. Together, our findings underline the role of maternal overnutrition on development and later life periods, especially in terms of metabolism and reproduction. References: 1. https://pubmed.ncbi.nlm.nih.gov/37665248/; https://pubmed.ncbi.nlm.nih.gov/34535697/

S10.1. MOLECULAR MECHANISMS OF SOCIAL HETEROGENEITY

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Sociability exists on a spectrum, and stable individual differences in sociability have been found in many animal species, including genetically similar laboratory mice. However, individual differences in social behavior will depend not only on the individual being tested but also on the partner's behavior and the specific relationship between the two. I will discuss our work using deep social phenotyping to examine how the behavior of all interaction partners influences social encounters in freely-moving mice. We use this method in combination with mass spectrometry to better understand the molecular mechanisms underlying social heterogeneity.

S10.2. THE MAINTENANCE OF THE SOCIAL BEHAVIOR: THE ROLE OF CENTRAL AMYGDALA

Hanna Trebesova

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The motivation behind social interaction can be attributed to a number of factors, including the pursuit of food or the formation of relationships with other individuals. Accordingly, this social interaction may be maintained, and the mechanism in question requires the involvement of different brain regions. The present study focuses on the central amygdala (CeA) and aims to elucidate whether the neural circuits involved in the initiation of social contact and in the maintenance of social interaction are identical or distinct. By employing optogenetic stimulation in association with a range of social stimuli, it is possible to observe changes in social interaction maintenance.

S10.3. SOCIABILITY AND ADDICTION-LIKE TRAITS IN MICE

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Alcohol dependence has a strong social component. Social factors like peer influence, societal norms, and stressors impact susceptibility to alcohol use. We use an automated rodent homecage that allows for continuous monitoring of mice living in a social environment. Our observations indicate that mice develop social bonds-like behavior. We observed a distinction between individuals in terms of their interactions as measured by Social Score. These moreover change over time. We measured alcohol consumption, motivation to alcohol, persistence in alcohol seeking and set an Addiction Score for each mouse. Our observations indicate a possible correlation between a Social Score and an Addiction Score.

S10.4. OXYTOCIN FACILITATES SOCIAL BEHAVIOR THROUGH INTERNEURONS IN THE RAT PREFRONTAL CORTEX

Alan Kania

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Oxytocin (OT) is widely studied for its profound pro-social effects, yet the mechanisms behind its diverse actions remain unclear[1,2]. Our project explores OT's role in the medial prefrontal cortex (mPFC). We demonstrated direct axonal projections from the hypothalamus to the mPFC and identified two types of oxytocin receptor (OTR) neurons activated during social interactions. Activating OTR+ axo-axonic interneurons in the mPFC enhances social behavior and shifts preferences, likely through selective modulation of principal neurons projecting to subcortical regions involved in social, reward, and anxiety-related processes, which may underlie the prosocial action of OT via the mPFC. References: [1] Froemke RC, et al. Oxytocin, neural plasticity, and social behavior. Annual Review of Neuroscience, 2021. doi: 10.1146/annurev-neuro-102320-102847 [2] Leng G, et al. Oxytocin—a social peptide? Deconstructing the evidence. Philos Trans R Soc Lond B Biol Sci., 2022. doi: 10.1098/rstb.2021.0055

S12.1. ROLE OF B CELLS AND CD8+ T CELLS IN GABAA RECEPTOR AUTOIMMUNE ENCEPHALITIS PATHOGENESIS

Nico Melzer

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GABAA-R encephalitis, a rare autoimmune condition, involves antibodies targeting neuronal gamma-aminobutyric acid A receptors. We analyzed CD8+ and CD4+ T cell receptor repertoires in a patient with this condition using next-generation sequencing and single-cell techniques. A highly expanded B cell clone in the cerebrospinal fluid was identified, producing antibodies that bind to GABAA-R, verified by ELISA and immunohistochemistry. Patch-clamp studies showed these antibodies alter synaptic inhibition, increasing cortical neuron excitability. Additionally, a clonally expanded CD8+ T cell population was found in the cere-

brospinal fluid and hippocampus, suggesting that neuron-targeting CD8+ T cells contribute independently to disease pathogenesis. References: 1. Brändle SM, et al. Cross-reactivity of a pathogenic autoantibody to a tumor antigen in GABAA receptor encephalitis. Proc Natl Acad Sci U S A., 2021. doi: 10.1073/pnas.1916337118 2. Bracher A, et al. An expanded parenchymal CD8+ T cell clone in GABAA receptor encephalitis. Ann Clin Transl Neurol., 2020. doi: 10.1002/acn3.50974

S12.2. CLINICAL PERSPECTIVE ON ANTI-IGLON5 DISEASE: BRIDGING AUTOIMMUNITY AND NEURODEGENERATION IN A NOVEL NEUROLOGICAL DISORDER

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Anti-IgLON5 disease is a newly recognized neurological condition that straddles the realms of autoimmunity and neurodegeneration. Patients typically experience a chronic and progressive course, characterized by gait instability, abnormal movements, bulbar dysfunction, and a unique sleep disorder involving obstructive sleep apnea and stridor. While the disease is defined by the presence of antibodies against IgLON5, a cell surface protein whose function is unclear, neuropathological investigations have identified an unusual tauopathy in the brainstem. The response to immunotherapy is generally poor, and delays in diagnosis, combined with the absence of progression biomarkers, hinder better outcomes. Understanding the disease's pathogenesis is essential for addressing its complexities. References: 1. Sabater L, et al. A novel non-rapid-eye movement and rapid-eye-movement parasomnia with sleep breathing disorder associated with antibodies to IgLON5: a case series, characterisation of the antigen, and post-mortem study. Lancet Neurol., 2014. Erratum in: Lancet Neurol. 2015. doi: 10.1016/S1474-4422(14)70051-1 2. Gaig C, Sabater L. New knowledge on anti-IgLON5 disease. Curr Opin Neurol., 2024. doi: 10.1097/WCO.0000000000001271

S12.3. APPLICATION OF ACTIVITY-BASED PROBES FOR VISUALIZING ALTERATIONS IN PROTEASOME AND IMMUNOPROTEASOME FUNCTION IN NEUROINFLAMMATION

Natalia Małek

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Proteostasis is essential for the regulation of protein synthesis, folding, and degradation within cells. When this balance is disrupted, it can lead to neurodegenerative diseases such as Alzheimer's and Parkinson's, which are marked by protein inclusions. Dysregulation often stems from altered catalytic activity of the 20S proteasome and immunoproteasome subunits. This study aimed to investigate how inflammation affects the activity of these subunits in human microglial cell lines. Our methods included transcriptomic analysis via RT-qPCR, proteomic analysis using Western blotting, and activity profiling with activity-based probes (ABPs). Results indicated increased expression of β1 and β5 subunits, along with altered activity levels, highlighting potential therapeutic targets for neurodegenerative conditions. References: 1. Malek N, Gladysz R, Stelmach N, Drag M. Targeting Microglial Immunoproteasome: A Novel Approach in Neuroinflammatory-Related Disorders. ACS Chem Neurosci. 2024 Jul 17;15(14): 2532-2544. doi: 10.1021/acschemneuro.4c00099. Epub 2024 Jul 6. PMID: 38970802; PMCID: PMC11258690. 2. Gladysz R, Malek N, Rut W, Drag M. Investigation of the P1' and P2' sites of IQF substrates and their selectivity toward 20S proteasome subunits. Biol Chem. 2022 Nov 16;404(2-3): 221-227. doi: 10.1515/hsz-2022-0261. PMID: 36376064.

S12.4. REGULATION OF INFLAMMATION RESOLUTION IN AGE-RELATED COGNITIVE DECLINE AND NEURODEGENERATIVE PATHOLOGY

Jakub Frydrych

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Neurodegenerative disease progression is closely associated with neuroinflammation, in which microglia are vital players. While short-term inflammation is beneficial and its controlled resolution facilitates repair and homeostasis, disruptions in the resolution of inflammation (RoI) can lead to chronic inflammation.

RoI is influenced by specialized pro-resolving mediators (SPMs), such as lipoxin A4 (LXA4), which activates the N-formyl peptide receptor-2 (FPR2). FPR2 is a versatile receptor that can trigger either pro-inflammatory or pro-resolving effects, depending on ligand structure. Our study examined age-related cognitive changes and RoI disturbances in wild-type and hAPPNL-F/NL-F KI male mice, revealing that aging impairs the availability of pro-resolving FPR2 ligands, leading to increased pro-inflammatory microglia polarization and FPR2 overactivation mediated by inflammatory ligands. These findings highlight FPR2 receptors as key regulators of the RoI process in age-related neurodegenerative pathology. References: 1. Trojan E, et al. The N-Formyl Peptide Receptor 2 (FPR2) Agonist MR-39 Improves Ex Vivo and In Vivo Amyloid Beta (1-42)-Induced Neuroinflammation in Mouse Models of Alzheimer's Disease. Mol Neurobiol., 2021. doi: 10.1007/s12035-021-02543-2 2. Trojan E, Frydrych J, Lasoń W, Basta-Kaim A. Prenatal stress increases the risk of the FPR2-related dysfunction in the brain's resolution of inflammation: a study in the humanized APPNL–F/NL-F mouse model of Alzheimer's disease. Curr Neuropharmacol., 2025. doi: 10.2174/011570159X345385241004060055

S13.1. SPATIAL INFORMATION ENCODING IN THE BRAIN: BEYOND NEURAL CELLS

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Animals encode information about their position in neuronal circuits of the hippocampus as patterns of spikes. This cellular representation of space is thought to be the basis for essential higher brain functions, including spatial navigation. However, whether this cellular representation of spatial information extends beyond neuronal circuits has long been unknown. In this talk, I will present experimental evidence obtained in head-fixed mice during virtual spatial navigation demonstrating that hippocampal astrocytes, a main type of non-neuronal cells, encode information about the animal's position in their intracellular calcium dynamics. Information encoded in astrocytes is complementary to that encoded in the spike output of nearby neurons. Moreover, perturbation of astrocytic calcium signals alters the coding properties of neuronal cells shaping their tuning functions and modulating their information content. Finally, I will discuss recent experimental findings showing spatial information encoding in astrocytic calcium signals recorded with miniaturized head-mounted two-photon microscopes (Mini2P) during freely moving spatial navigation. These findings challenge current models of brain information processing and advocate for the inclusion of an additional non-neural reservoir of information in the conceptualization of the network mechanisms that support how brains encode and process spatial information.

S13.2. MAPPING SPATIAL ORGANIZATION OF FUNCTIONAL INPUTS IN VALENCE-RELATED AMYGDALO-HIPPOCAMPAL CIRCUITS

Andrea Barberis, Dario Cupolillo, Vincenzo Regio

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The formation of memories in response to aversive or rewarding stimuli is crucial in guiding avoidance or approach behaviors. Scattered, projection-defined neuronal populations within the basolateral amygdala (BLA) selectively activate during encoding and retrieval of memories associated with either positive or negative valence. Interestingly, BLA neurons projecting to the CA1 area of ventral hippocampus (vCA1) respond to both positive or negative predicting cues with no marked bias, suggesting that, within the whole responding population, two distinct subnetworks relay opposite information to vCA1. However, the mechanism by which vCA1 pyramidal neurons discern between positive and negative-related information remains unclear. The valence information might stay segregated within two distinct neuronal populations in vCA1, or it might also converge onto the same vCA1 neurons, which have the capability to specifically encode negative or positive valence. We suggest that valence-activated BLA neurons contact vCA1 dendrites in a precise spatial organization that together with inhibitory synapses can generate unique valence-related spiking patterns in the postsynaptic neuron. To validate this hypothesis, we aimed at building a map of the spatial location of functional synaptic inputs from BLA, vCA3 and bistratified interneurons onto vCA1 pyramidal neurons. To this end, we have developed an automated procedure to perform single-spine calcium imaging in the whole

vCA1 dendritic arbor exploiting custom made neural network algorithms combined with electrophysiology and optogenetics. This integrated approach allowed to reveal the unique distribution of BLA and vCA3 and inhibitory inputs onto the whole dendritic arbor of vCA1 pyramidal neurons.

S13.4. THE MOLECULAR MECHANISMS OF INTERSYNAPTIC CO-PLASTICITY: HOW EXCITATORY AND DIFFERENT INHIBITORY SYNAPSES SHAPE EACH OTHER

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Far from being static, GABAergic synapses exhibit a remarkable capacity for plasticity, reshaping neural circuits to fine-tune brain function. Yet, the rules that govern inhibitory plasticity and its interaction with excitatory synapses remain unclear. Given the diversity of hippocampal interneurons, it is likely that inhibitory plasticity follows unique, synapse-specific mechanisms that have yet to be fully uncovered. In our experiments, we discovered a form of communication between excitatory and inhibitory plasticity, which we call co-plasticity. The way these synapses change together depends on where the inhibitory input is located on pyramidal neurons. Co-plasticity follows different patterns at the soma, the proximal dendrites in the CA1 stratum radiatum, and the distal dendrites in the stratum lacunosum-moleculare. We also found that the extracellular matrix acts as a gatekeeper, restricting certain forms of inhibitory plasticity—when it is removed, hidden GABAergic plasticity emerges. Furthermore, we showed that maintaining inhibitory long-term potentiation (iLTP) relies on a key transsynaptic interaction between neuroligin-2 and neurexin. Interfering with this adhesion abolished already induced GABAergic plasticity, demonstrating that plastic changes at inhibitory synapses also exhibit a late phase. Finally, we found that naturally occurring neurosteroids, such as allopregnanolone and pregnanolone sulfate, act as metaplastic regulators, shaping the balance between excitatory and inhibitory plasticity. Together, these findings provide new insights into the molecular and structural mechanisms regulating inhibitory plasticity and its coordination with excitatory synaptic changes, highlighting its relevance for experience-dependent circuit remodeling.

FINANCIAL SUPPORT: This work was supported by National Science Centre, Poland NCN grant 2021/43/B/NZ4/01675.

S14.1. THE SHAPES AND STATES OF CNS MACROPHAGES IN THE HEALTHY AND DISEASED HUMAN BRAIN

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Microglia exhibit a unique transcriptional profile that reflects their role as macrophages in the central nervous system (CNS). These cells are often key contributors to the initiation and progression of neuroinflammatory and neurodegenerative diseases. Recent studies have shed light on the changes microglia undergo during development and in various CNS conditions. Advances in single-cell and spatial profiling technologies have enabled detailed phenotyping of individual microglia. This discussion will focus on the heterogeneity of microglia and their critical roles in human CNS development, maintaining homeostasis, and contributing to neuroinflammatory diseases. References: 1. Alsema AM, et al. Decoding spatial gene activity changes in multiple sclerosis lesion progression, Nature Neuroscience, in press 2. Eggen BJL, How the cGAS-STING system links inflammation and cognitive decline. Nature., 2023. doi: 10.1038/d41586-023-02240-1

S14.2. INTEGRATING SINGLE-CELL SEQUENCING AND OLINK PROTEOMICS IN UNDERSTANDING IGLON5 DISEASE

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Recent research on IgLON5 disease has utilized single-cell sequencing and OLINK proteomics to gain insights into its pathophysiology. Single-cell analysis enables the characterization of individual immune and neuronal cell populations, revealing the heterogeneity of responses associated with IgLON5 antibodies. OLINK

technology provides a comprehensive profiling of proteins in the cerebrospinal fluid, identifying potential biomarkers linked to disease progression and severity. Together, these advanced techniques have facilitated a deeper understanding of the immune and neurodegenerative processes underlying IgLON5 disease. This integrated approach may pave the way for the development of targeted therapies and improved diagnostic strategies for affected patients. References: 1. Graus F, et al. A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol., 2016. doi: 10.1016/S1474-4422(15)00401-9 2. Gelpi E, et al. Neuropathological spectrum of anti-IgLON5 disease and stages of brainstem tau pathology: updated neuropathological research criteria of the disease-related tauopathy. Acta Neuropathol., 2024. doi: 10.1007/s00401-024-02805-y

S14.3. ASSESSING BLOOD-BRAIN BARRIER INTEGRITY AND NEUROINFLAMMATION IN NEURODEGENERATIVE DISEASE USING MASS CYTOMETRY

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The blood-brain barrier (BBB) serves as a critical interface between the brain tissue and peripheral blood, regulating the transport of metabolites and nutrients. Since vascular damage can be an early indicator of Alzheimer's disease (AD), this study aimed to investigate the role of the periodontopathogen Porphyromonas gingivalis in BBB disruption and AD progression. Brain tissues from C57BL/6J and 5xFAD mouse models infected orally with P. gingivalis were analyzed using mass cytometry with a panel of 40 antibodies. Results showed increased neuroinflammation and reactive gliosis in the infected mice's brain tissue, suggesting a link between P. gingivalis infection, BBB breakdown, and AD. References: 1. Dominy SS, et al. Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv., 2019. doi: 10.1126/sciadv.aau3333

S14.4. DISCOVERY OF YOLKIN PEPTIDE COMPLEX: A NEW FRONTIER IN BRAIN HEALTH AND IMMUNE MODULATION

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Scientific interest in food-derived compounds for preventing and managing neurodegenerative diseases has grown, with egg yolk identified as a rich source of bioactive peptides. The yolkin peptide complex exhibits immunomodulatory and neuroprotective properties, regulating macrophage activity, enhancing immune responses, and supporting neuronal function through neuroprotection and regeneration. These effects highlight its potential for treating neurodegenerative and immune-related disorders. This presentation will explore yolkin's discovery, complex characterization, and immunoregulatory and neuroprotective properties. Understanding yolkin's significance offers insights into its potential role in protecting against neurodegenerative diseases. Additionally, its ability to support neuroprotection and immune balance may contribute to healthy ageing by preserving cognitive function and overall well-being. References: 1. Zambrowicz A, Zabłocka A, Bednarz D, Bobak Ł . Importance for humans of recently discovered protein compounds – yolkin and yolk glycopeptide 40, present in the plasma of hen egg yolk. Poult Sci. 2023 Jul;102(7): 102770. doi: 10.1016/j.psj.2023.102770. 2. Kazana W, Jakubczyk D, Siednienko J, Zambrowicz A, Macała J, Zabłocka A. Mechanism of Molecular Activity of Yolkin-a Polypeptide Complex Derived from Hen Egg Yolk-in PC12 Cells and Immortalized Hippocampal Precursor Cells H19-7. Mol Neurobiol. 2023 May;60(5): 2819-2831. doi: 10.1007/s12035- 023-03246-6.

S15.1. CLOCK AND BRAIN HEALTH – IMPORTANT LESSON FROM FLIES

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Parkinson's disease (PD) is one of the most common neurodegenerative disorder, caused by both genetical and environmental factors. Among the symptoms of PD are sleep problems, which affect quality of life and daytime functioning. Several lines of evidence suggest that one cause of sleep problems in patients with PD is circadian disfunction, which can accelerate the neurodegenerative process. However, the cellular mechanisms linking the circadian clock to neurodegeneration are still poorly understood. In this study we will show how PD progression affects clock functioning, and how clock disruption may enhance PD development. References: 1. Szypulski K, et al. Autophagy as a new player in the regulation of clock neurons physiology of Drosophila melanogaster. Sci Rep. 2024. doi: 10.1038/s41598-024-56649-3 2. Doktór B, et al. Effects of MUL1 and PARKIN on the circadian clock, brain and behaviour in Drosophila Parkinson's disease models. BMC Neurosci., 2019. doi: 10.1186/s12868-019-0506-8

S15.2. GLIA-NEURON INTERACTIONS MEDIATED BY VESICULAR DEGRADATION PATHWAYS AFTER TRAUMATIC NERVOUS SYSTEM INJURY IN DROSOPHILA

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We are interested in membrane trafficking pathways in glia, especially phagocytosis, early damage signalling and their involvement in neuronal death. We take advantage of Drosophila as a genetically tractable model and inflict injuries using simple paradigms. We uncovered a role for glial LC3-associated phagocytosis in axon debris clearance1 and found elevated glial immunity in the absence of LAP. We deciphered how STAT TF activity is set during glial reactivity by degradation of a repressor2. We aim to understand the adaptive changes in glia elicited via such vesicular pathways in face of damage. References: 1. Szabó Á, et al. LC3-associated phagocytosis promotes glial degradation of axon debris after injury in Drosophila models. Nat. Commun., 2023. doi: 10.1038/s41467-023-38755-4 2. Vincze V, et al. Selective autophagy fine-tunes Stat92E activity by degrading Su(var)2-10/PIAS during glial injury signalling in Drosophila. bioRxiv, 2024. doi: 10.1101/2024.08.28.610109

S15.3. TRMT2A INHIBITION, A CAUSATIVE TREATMENT OF POLYGLUTAMINE DISEASES? THE PATH FROM BASIC FLY SCIENCE TOWARDS DRUG DEVELOPMENT

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Genes linked to polyglutamine (polyQ) diseases like Huntington's disease share expanded CAG stretches within the coding region, translated into expanded glutamine tracts in disease-linked proteins. These polyQ stretches are causative for disease. Due to their dominant inheritance and monogeneticity, diagnosis prior disease onset is common, but no cure is available so far. We show that inactivation of the tRNA-methyl transferase 2 homolog A (TRMT2A) suppresses polyQ-induced toxicity and aggregation in yeast, flies, HEK cells and mice. As loss of TRMT2A in analyzed organisms did not result in phenotypic abnormalities, inhibition of TRMT2A represents a therapeutic avenue to treat polyQ diseases. References: 1. Witzenberger M, et al. Human TRMT2A methylates tRNA and contributes to translation fidelity. Nucleic Acids Res., 2023. doi: 10.1093/nar/gkad565 2. Voßfeldt H, et al. Large-scale screen for modifiers of ataxin-3-derived polyglutamine-induced toxicity in Drosophila. PLoS One., 2012. doi: 10.1371/journal.pone.0047452

S15.4. STRESS SHAPES BRAIN FITNESS IN THE PROGENY THROUGH EPIGENETIC MODIFICATIONS

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Glioblastoma is characterized by rapid proliferation, extensive invasiveness, and resistance to current therapeutic interventions. Despite similar genetic mutations, patients exhibit considerable heterogeneity in survival outcomes, ranging from weeks to years. This talk introduces a novel perspective on the interaction between the tumor and host, influenced by paternal environmental or lifestyle factors, termed the „brain fitness hypothesis." Continuous light exposure induces oxidative stress in the brains of F1 progeny, which compromises brain fitness and accelerates tumor growth, leading to sex-dependent differences in life expectancy. Among the heritable mechanisms, miRNAs has emerged as a key mediator in the transmission of parental environmental exposure to subsequent generations, enhancing the propensity of glioblastoma cells to proliferate and extend tumor microtubes. Consequently, accelerated glioblastoma progression is driven by heritable risk factors acquired through stress exposure in the parental generation. References: 1. Portela M, et al. Glioblastoma cells vampirize WNT from neurons and trigger a JNK/MMP signaling loop that enhances glioblastoma progression and neurodegeneration. PLoS Biol., 2019. doi: 10.1371/journal.pbio.3000545 2. Losada-Pérez M, et al. Synaptic components are required for glioblastoma progression in Drosophila. PLoS Genet., 2022 doi: 10.1371/journal.pgen.1010329

POSTERS

P1.01. DEEP BODY PEPTIDERGIC AFFERENTS LACK ADVILLIN EXPRESSION: IMPLICATIONS FOR SENSORY NEURON PROFILING

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INTRODUCTION: Heterogenous population of peripheral neurons is housed in dorsal root ganglia (DRG). Those diverse somatosensory afferent neurons mediate key bodily sensations ranging from touch and proprioception to temperature and pain. Advillin (Avil), an actin-binding protein, is a commonly used marker of all sensory and sympathetic neurons. However, recent findings question its universality, particularly in certain peptidergic subpopulations.

AIM(S): To evaluate which afferents are not covered by Avil and thus could exhibit new interesting population of sensory neurons.

METHOD(S): We used three transgenic mouse lines: Avil-eGFP labelling majority of sensory and sympathetic neurons, Calca-eGFP labelling peptidergic nociceptors, and ChRNA3-eGFP labelling sympathetic neurons and 'silent nociceptors', a unique subpopulation of peptidergic nociceptors. Sensory neurons innervating discreet bodily compartments (i.e. tibia, knee, bladder, hind paw) were retrogradely labelled with Fast Blue. Lumbar and sacral DRG were cryosectioned and immunostained for sensory markers (i.e., CGRP, Avil, Tubulin-βIII, IB4). Selected adrenoceptors were analysed in situ with RNAscope assay also. Following confocal microscopy, detailed colocalization analysis was performed. Additionally, representative DRG were optically cleared using PACT protocol and imaged with confocal and light-sheet microscopes. Image analysis was done in Fiji and Imaris, while quantification data was analysed by custom Python scripts and GraphPad Prism.

RESULTS: We demonstrate that Avil does not universally cover sensory neurons. Specifically, a subset of peptidergic afferents enriched in CGRP and innervating deep tissues exhibits low or undetectable levels of Avil. Data is supported by *post hoc* snRNAseq analysis.

CONCLUSIONS: Avil is not a universal marker of sensory neurons, biased towards superficial skin afferents.

FINANCIAL SUPPORT: Funded by National Science Centre grant (2022/D/NZ4/02676), held by M. Kucharczyk.

P1.02. PIEZO2-DEPENDENT RAPID PAIN SYSTEM IN HUMANS AND MICE

Marek Brodzki[1], Otmane Bouchatta[1], Houria Manouze[1], Gabriela B. Carballo[1], Huasheng Yu[2], Emma Kindström[1], Felipe M. de- Faria[1], Oumie Thorell[1,3], Jaquette Liljencrantz[4,5], Christoffer Karlsson[1], Melisa B. Maidana Capitán[1], Kevin K. W. Ng[1], Katarzyna Terejko[1,6], Max Larsson[1], Wenqin Luo[2], Andrew G. Marshall[1,7], Alexander T. Chesler[4,8], Håkan Olausson[1], Saad S. Nagi[1,3], Marcin Szczot[1]

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INTRODUCTION: Mechanical pain in humans manifests in diverse forms, yet current knowledge of cutaneous nociceptor classes does not fully explain this variety. One sensation that is particularly understudied is the pain evoked by hair pulling.

AIM(S): To identify and characterize the neural mechanisms underlying hair-pull pain in humans and determine the role of PIEZO2 and specific nociceptor types in this process.

METHOD(S): We studied individuals with PIEZO2 deficiency syndrome psychophysically, performed single-unit axonal recordings, pharmacological mapping, and anatomical tracing. Functional and anatomical imaging in mice as well as behavioral studies in humans,

including Aβ-deafferented individuals and nerve block experiments, were also conducted.

RESULTS: Hair-pull pain was absent in PIEZO2-deficient individuals, indicating its dependence on PIEZO2. Nerve recordings identified a class of rapidly conducting, cooling-sensitive nociceptors selectively responsive to hair-pull stimuli. These were pharmacologically linked to a distinct transcriptomic class and shown to be myelinated and associated with hair follicles. In mice, Piezo2 was essential for robust activation of homologous nociceptors. In humans, hair-pull stimuli triggered a unique nocifensive response and required Aβ-fiber input for pain perception.

CONCLUSIONS: We reveal a previously unrecognized class of human nociceptors specialized for hair-pull pain, defined by distinct molecular, anatomical, and functional properties, and dependent on PIEZO2 and Aβ-fiber signaling.

FINANCIAL SUPPORT: Swedish Research Council Starting Grant no. 2020-01107; Knut and Alice Wallenberg Foundation Fellowship; Swedish Research Council Project Grant no. 2021-03054; Knut and Alice Wallenberg Project Grant no. 2019.0047; Knut and Alice Wallenberg Clinical Scholar Grant no. 2019.0487; ALF Grants Region Östergötland; Swedish Society of Medicine Project Grant; Magnus Bergvalls Stiftelse Research Grant; Western Sydney University Funding Scheme; Intramural Research Program of the NIH, specifically the NCCIH.

P1.03. BEHAVIOURAL, ELECTROPHYSIOLOGICAL AND HISTOLOGICAL CHANGES IN A NEW MODEL OF ANOSMIA

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INTRODUCTION: Changes in the sense of smell are associated with cognitive decline and major neurological disorders, both linked to disrupted brain rhythms. Nasal respiration not only supports odor detection but also entrains neural activity across widespread brain regions, including corticolimbic circuits, playing a role in learning and memory processes.

AIM(S): This study's aim was to explore how input from the NE affects electrical activity patterns.

METHOD(S): We developed a novel, reversible model of anosmia in rats by infusing gadolinium into the nares. Olfactory function was assessed over time. Local field potentials (LFP) were recorded from the olfactory bulb, ventral striatum, and prefrontal cortex during wakefulness and slow-wave sleep. Immunohistochemical analysis measured olfactory sensory neuron integrity via olfactory marker protein (OMP) expression.

RESULTS: Gadolinium infusion induced a significant reduction in olfactory function lasting up to two weeks, with gradual recovery thereafter. During wakefulness, LFP recordings from the olfactory bulb showed reduced amplitude in the respiration-related rhythm (1–10 Hz) and gamma band (30–90 Hz) in treated animals. Stronger respiration-locked olfactory bulb activity correlated with better olfactory performance across all subjects. Anosmia was also associated with decreased 1–10 Hz power in the ventral striatum and prefrontal cortex, and diminished gamma oscillations in the ventral striatum. These neural disruptions were specific to wakefulness; slow-wave sleep activity remained unaffected. Immunohistochemistry confirmed a loss of olfactory sensory neurons, indicated by decreased OMP expression in treated rats.

CONCLUSIONS: This reversible anosmia model demonstrates disrupted brain oscillations during wakefulness in key brain regions involved in cognition. These results suggest that altered neural rhythms are a direct consequence of anosmia and may contribute to the cognitive impairments linked to impaired olfactory processing.

FINANCIAL SUPPORT: Narodowe Centrum Nauki.

P1.04. CHANGES IN SYNAPTIC TRANSMISSION FROM MUSCLE SPINDLES TO MOTONEURONS IN RESPONSE TO ENDURANCE TRAINING

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INTRODUCTION: Membrane and firing properties of motoneurons (MNs) are modified due to increased motor activity. However, it remains unknown whether adaptations also concern peripheral input from muscle receptors, most of all muscle spindles, which are a potent source of excitatory connections to MNs.

AIM(S): The aim of this study in a rat model was to investigate whether physical exercises during treadmill endurance training evoke adaptive changes in Ia afferent synaptic transmission to MNs.

METHOD(S): Male Wistar rats (n=15) were exposed to a 5-week endurance running on a treadmill, and a day after the last training session an acute electrophysiological experiment was performed on each rat under general anesthesia. The respective control group of untrained rats (n=15) was assigned. Lumbar spinal MNs innervating the medial gastrocnemius (MG) or lateral gastrocnemius and soleus (LG-S) muscles were investigated intracellularly. The passive membrane properties and parameters of monosynaptic Ia EPSPs evoked from homonymous or heteronymous afferents from synergistic muscles were compared between the experimental and control groups.

RESULTS: The potentiation of Ia synaptic excitation of motoneurons, expressed by higher heteronymous EPSP amplitudes was observed in slow-type MNs, both in MG and LG-S MNs, and positive correlation of EPSP amplitudes with input resistance was observed. No significant changes in EPSP parameters were found for homonymous EPSPs.

CONCLUSIONS: The demonstrated adaptations to endurance training might be attributed to a greater size and/or number of Ia synapses on MNs, changes in MN membrane properties or altered levels of presynaptic inhibition of Ia fibers. Selective enhancement of synaptic transmission to slow MNs is likely related to the greater recruitment of slow motor units during treadmill exercises.

FINANCIAL SUPPORT: The study was supported by the National Science Centre (NCN) Grant No. 2022/45/B/NZ7/00102.

P1.05. CHANGES IN SYNAPTIC TRANSMISSION FROM MUSCLE SPINDLES TO MOTONEURONS IN RESPONSE TO WEIGHT-LIFTING TRAINING

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INTRODUCTION: Long-lasting physical exercises evoke adaptive changes in electrophysiological properties of motoneurons (MNs). It remains unknown whether such adaptations also concern peripheral input from muscle receptors, most of all muscle spindles, which provide powerful excitatory input to MNs.

AIM(S): The aim of this study was to investigate whether the strength training based on weight-lifting exercises evokes changes in afferent synaptic transmission to MNs.

METHOD(S): Male Wistar rats (n=15) performed a 5-week voluntary progressive weight-lifting training, and a day after the last training session an acute electrophysiological experiment was performed under general anesthesia. The respective control group was composed of rats restricted to standard cage activity (n=15). Intracellular recordings were made from two pools of MNs: innervating the medial gastrocnemius (MG, n=188) or lateral gastrocnemius and soleus (LG-S, n=187) muscles. Basic membrane properties of MNs were measured and monosynaptic Ia EPSPs were recorded after stimulation of homonymous or heteronymous afferents from synergistic muscles.

RESULTS: EPSP amplitudes were higher in the weight-lifting group in comparison to control. This effect was observed for homonymous EPSPs as well as for heteronymous EPSPs in fast-type MNs. The increase in the input resistance was also observed in fast MNs in response to the training, both in MG and LG-S MNs, and this membrane property positively correlated with the EPSP amplitude.

CONCLUSIONS: The enhancement of synaptic transmission from muscle spindles to MNs in response to weight-lifting training suggests synaptic plasticity, but one should also consider adaptive changes in MN membrane properties or altered levels of presynaptic inhibition of Ia fibers. Adaptive changes were predominant in fast MNs, suggesting their potent recruitment during weight-lifting training.

FINANCIAL SUPPORT: The study was supported by the National Science Centre (NCN) Grant No. 2022/45/B/NZ7/00102.

P1.06. THE IMPACT OF ANODAL AND CATHODAL TSDCS ON PASSIVE MEMBRANE AND FIRING PROPERTIES OF SPINAL MOTONEURONS IN SOD1 G93A MICE

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INTRODUCTION: In Amyotrophic Lateral Sclerosis (ALS), the electrophysiological profile of spinal motoneurons (MNs) undergoes drastic alterations, marking the hallmark for their degeneration. Transcutaneous spinal direct current stimulation (tsDCS) is a neuromodulation method that evokes long-term neuroplasticity in MNs. We have recently demonstrated that chronic tsDCS alters spinal MN synaptic excitation levels in a polarity-dependent manner.

AIM(S): Here, we expand our investigations to determine if spinal MNs' membrane, threshold, and firing properties are also affected by chronic tsDCS.

METHOD(S): Presympthomatic p35-p40 SOD1 G93A mice were exposed to anodal, cathodal, or sham tsDCS of 60 μA for 15 minutes for 10 days under isofluorane anesthesia. In vivo intracellular recordings of MNs were performed at p45-p50, and the MNs' electrophysiological profile was analysed.

RESULTS: Following anodal tsDCS, a significant reduction in the MNs' plateau input resistance and firing gain was observed. Conversely, cathodal tsDCS did not change MNs' input resistance but increased their membrane time constant and reduced the rheobase and Ion current. Surprisingly, both anodal and cathodal tsDCS increased the MNs' SAG ratio. The polarization-dependent changes in the electrophysiological profile of MNs significantly influenced their population behavior. After anodal polarization, a notably larger proportion of MNs were able to reach a primary range of firing in response to depolarizing ramps of current. In contrast, following cathodal polarization, more cells could generate action potentials during ramp current injection but without reaching the primary firing range.

CONCLUSIONS: These results highlight the differential effects of 10 days of anodal and cathodal tsDCS on MN electrophysiological profile in ALS.

FINANCIAL SUPPORT: This research was supported by Polish National Science Centre grants 2019/35/B/NZ4/02058 and 2022/04/Y/NZ4/00117.

P1.07. THE EFFECTS OF ELECTRODE CONFIGURATION ON MNS' RESPONSE TO TSDCS

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INTRODUCTION: Trans-spinal direct current stimulation (tsDCS) is a non-pharmacological neuromodulatory technique evoking functional changes in spinal circuit activity. However, the effects of tsDCS are highly variable between interventions, and the application parameters responsible for its effectiveness remain largely unknown. It was recently suggested that the tsDCS electrode configuration, rather than current dose, plays a crucial role in producing the neuromodulatory effects.

AIM(S): This study aims to establish the impact of electrode arrangement on tsDCS effects on passive membrane, threshold, and firing properties of spinal motoneurons (MNs).

METHOD(S): Due to the invasive nature of the investigations, all experiments were performed on B6SJL WT mice under general anesthesia. 15 minutes of anodal or cathodal tsDCS of 60 uA was applied to the mice in rostro-caudal (RC – two rectangular electrodes placed on the animal's back) or dorso-ventral (DV – rectangular electrode on the back, and crocodile clip attached diagonally to the abdominal skin) arrangement. In vivo intracellular recordings of spinal MNs were performed before and up to 1h after tsDCS application.

RESULTS: In both setups, cathodal tsDCS was the most prominent in affecting MNs' electrophysiological properties. In RC, it led to an increase in MNs' peak and plateau input resistance, subthreshold ion channel activity, and firing frequency gain. In the DV arrangement, it caused more modest changes, increasing MNs' peak and plateau RIN, and membrane time constant. On the other hand, anodal tsDCS tended to increase MNs' input resistance only in the RC setup.

CONCLUSIONS: These results indicate that different electrode configurations during tsDSC application lead to diversified alterations in MNs' intrinsic excitability and firing properties. Cathodal tsDCS in the RC configuration evokes the strongest alterations in MNs' passive membrane and firing properties.

FINANCIAL SUPPORT: NCN 2022/04/Y/NZ4/00117.

P1.08. CONCOMITANT EFFECTS OF FATIGUE AND POTENTIATION IN RAT MEDIAL GASTROCNEMIUS FAST MOTOR UNITS

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INTRODUCTION: During contractile activity of muscles the force of their motor units is unstable. Namely, the force can be reduced (i.e., fatigue/performance fatigability) or increased (i.e., potentiation) in a stimulation frequency and task-dependent manner, through the parallel action of various potentiating and fatiguing mechanisms. These two effects can be observed concomitantly, with the most characteristic example being increased submaximal force in parallel with maximal tetanic force reduction.

AIM(S): The majority of these observations come from isolated whole muscle models, with less data available at the motor unit level. Therefore, the study aimed to investigate and compare the dynamics of the development of force potentiation and fatigue in both types of fast motor units.

METHOD(S): We investigated in situ the dynamics of fatigue and potentiation development in fast motor units of rat medial gastrocnemius, fast fatigable (FF) and fast fatigue resistant (FR) ones, using a 180-s repetitive isometric contractile protocol including twitches, unfused tetani of different fusion degrees (30 Hz, 40 Hz) and maximal tetanic contractions (150 Hz).

RESULTS: Potentiation of twitch and unfused tetanic force was apparent in both motor unit types in parallel with reduced maximal tetanic force (stronger for FF motor units). The potentiation depended on the fusion degree and was the strongest for moderately fused tetanic contractions at 30 Hz. Considerable overlap in potentiation magnitude between FF and FR units was observed, across stimulation frequencies. However, FF units exhibited potentiation more rapidly compared to FR units.

CONCLUSIONS: These observations directly indicate that force potentiation and fatigue coexist in both types of fast motor units most probably due to different intracellular mechanisms. Furthermore, they illustrate the progressively narrowing range of possibilities for regulating fast motor unit force by changing the stimulation frequency during activity.

P1.09. THE ROLE OF CAP2 IN THE NEUROMUSCULAR SYSTEM

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INTRODUCTION: The neuromuscular system controls voluntary movement through signals from motoneurons to muscles. This process relies not only on synaptic integrity but also on proper cytoskeletal organization. Actin dynamics and its regulators, such as cyclase-associated protein 2 (Cap2), play a key role in the neuromuscular system . However, the function of Cap2 at the neuromuscular junctions (NMJ) remains unknown.

AIM(S): To understand the role of Cap2 at the neuromuscular system.

METHOD(S): We employed immunohistochemistry, muscles cross-sectioning, and confocal microscopy to investigate NMJ morphology and muscle histology in tissue-specific KO mice.

RESULTS: Muscle-specific Cap2 knockout mice show particularly severe phenotypes, with male mutants exhibiting stunted growth and premature death. Skeletal muscle cross-sections analysis reveals abnormal morphology and actin accumulation, suggesting impaired cytoskeletal organization. These changes appeared in both fast- and slow-twitch muscles, highlighting the essential role of Cap2 in muscle maintenance. At the NMJ, Cap2 loss in systemic KO mice leads to structural abnormalities, such as an increased number of NMJs with atypical sizes, postsynaptic AChR fragmentation, and presynaptic axonal swelling. While motoneuron-specific Cap2 knockouts show presynaptic changes, muscle-specific deletion causes extensive postsynaptic disruption and altered NMJ size, emphasizing the importance of Cap2 in maintaining neuromuscular integrity through its function in the postsynaptic machinery.

CONCLUSIONS: Our findings indicate that Cap2 is essential for the proper function and structural integrity of the neuromuscular system. Its disruption leads to widespread impairments affecting both muscle tissue and neuromuscular synapses. These results highlight the critical role of Cap2 in maintaining overall neuromuscular health and stability.

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P1.10. SH3BP2 – A NEW REGULATOR OF NEUROMUSCULAR SYNAPSES

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INTRODUCTION: The neuromuscular junction (NMJ) plays a critical role in muscle function, yet the molecular mechanisms governing its development and maintenance remain incompletely understood. Dysfunction of these synapses is linked to severe genetic and autoimmune disorders, highlighting the need to identify key regulatory components. We identified SH3BP2 as a potential interactor of αDB1, but its localization and function in skeletal muscles remain unexplored.

AIM(S): The aim is to determine the function of SH3BP2 in skeletal muscles.

METHOD(S): We used protein interaction analysis (peptide pull-downs, co-immunoprecipitation, mass spectrometry) combined with functional analysis using siRNA knock-down in C2C12 myotubes and muscle-specific SH3BP2 knockout in mice. NMJ morphology was assessed using confocal and electron microscopy. Additionally muscle performance and electrophysiological recordings were analyzed in the study.

RESULTS: Our experiments identified that SH3BP2 is strongly concentrated at the NMJ where, in a polyvalent way, it interacts with the dystroglycan complex (DGC) and Acetylcholine receptor (AChR) pentamers. We demonstrated that SH3BP2 works as a scaffold clustering AChR. On the mechanistic level, SH3BP2 self-interacts organizing high molecular mass dynamic protein droplets that recruit AChR molecules. Consistently, muscle-specific SH3BP2 KO leads to NMJ fragmentation, abnormal ultrastructure, impaired synaptic transmission, decreased muscle strength and physical fitness of mutant mice.

CONCLUSIONS: We identify SH3BP2 as a novel postsynaptic regulator at the NMJ. This scaffold protein through polyvalent molecular interactions and phase separation-dependent process contributes to AChR clustering. Further studies should address if SH3BP2 plays similar functions in the brain.

FINANCIAL SUPPORT: This work has been supported by the Polish National Science Centre, grants: 2016/21/B/NZ3/03638 and 2020/39/D/NZ5/02004 and German Research Council grants HA3309/7-1.

P1.11. THE ROLE OF DOPAMINE-SENSITIVE MOTOR CORTICAL CIRCUITS IN THE DEVELOPMENT AND EXECUTION OF SKILLED FORELIMB MOVEMENTS

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INTRODUCTION: The primary motor cortex (M1) plays a key role in controlling voluntary movements and is innervated by dopaminergic fibers. There is growing evidence that dopamine (DA) transmission in M1 is crucial for motor skill learning.

AIM(S): Nevertheless, the spatiotemporal DA dynamics and activity patterns of DA-sensitive neuronal populations in the M1, during the formation and execution of skilled forelimb movements, have not yet been investigated.

METHOD(S): We have developed a forelimb-specific joystick task for head-fixed animals and used fiber photometry to track DA dynamics and population-level calcium (Ca^{2+}) activity in the M1 of D1Cre and D2Cre mice, during the development of skilled behavior, and following subsequent changes in reward threshold and contingency. Furthermore, we determined the laminar distribution of D1 receptor-positive (D1+) and D2 receptor-positive (D2+) cells in the M1, employed retrograde tracings to determine their long-range connections, and used reversible optogenetic inhibition to investigate their functional contribution to motor performance.

RESULTS: We found that DA release events and Ca^{2+} transients were temporally associated with joystick movements and reward consumption. Moreover, DA dynamics and the population-level activity of DA-receptive neurons scaled with the vigor of forelimb movements and tracked the relationship between actions and their outcomes. We showed that D1+ and D2+ neurons have a discrete distribution in the layers of the M1, with D1+ neurons primarily found in the deep lay-

ers, and D2+ cells distributed in the superficial layers. We also showed that only a small fraction of D1+ and D2+ projection neurons of the M1 contact long-range targets. Finally, we found that when cortical inhibition was released, the number of rewarded joystick movements in D1Cre and D2Cre animals decreased.

CONCLUSIONS: Overall, our findings show how phasic DA signals in the M1 facilitate reinforcement motor learning of skilled behavior.

FINANCIAL SUPPORT: National Science Centre, Poland – SONATA 2020/39/D/NZ4/00503.

P1.12. EFFECTS OF LATERALIZED VISUAL STIMULATION ON MIDBRAIN DOPAMINERGIC NEURON ACTIVITY AND STRIATAL DOPAMINE RELEASE DYNAMICS

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INTRODUCTION: Ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), plays a vital role in motor control and decision-making via dopamine release in the striatum. Asymmetries in striatal dopamine levels can bias action selection, leading to lateralized behaviours such as orienting or approach/withdrawal responses. Dopaminergic (DA) neurons integrate directional environmental cues, with the superior colliculi (SC) serving as a key sensory relay, particularly for visual input. While most studies have focused on the dominant, ipsilateral SC projections to the VTA/SNc, both mono- and polysynaptic contralateral connections exist, potentially contributing to lateralized dopaminergic responses.

AIM(S): We set out to characterise responses of DA neurons in the VTA/SNc to lateralized eye stimulation and investigate the resulting changes in striatal dopamine release on both sides of rat brain. We hypothesized that DA neuron responses depend on which eye receives the stimulus, leading to asymmetrical striatal dopamine release.

METHOD(S): To test our hypothesis in vivo extracellular single-unit recordings combined with SC disinhibition, and uniocular light stimulation were performed. Fiber photometry was employed to measure dopamine release in the striatum. We also conducted behavioural tests to assess the impact of optogenetic stimulation of the investigated pathway on orienting behaviours in rats.

RESULTS: Electrophysiological recordings revealed that uniocular stimulation predominantly evoked excitatory responses in DA neurons within the VTA/SNc. This effect showed a significant interaction with eye of stimulation, but no clear hemispheric asymmetry was observed. Fiber photometry revealed higher dopamine release in the striatum contralateral to the stimulated eye, compared to the ipsilateral site.

CONCLUSIONS: Our data suggest that lateralized sensory input can differentially modulate dopaminergic activity and striatal signalling, with implications for understanding sensory-driven action selection and motor control.

FINANCIAL SUPPORT: The National Science Centre, OPUS 17 2019/33/B/NZ4/03127.

P1.13. TIME NECESSARY TO RESTITUTE EXTRA INITIAL FORCE IN FAST MOTOR UNITS IN RAT MEDIAL GASTROCNEMIUS MUSCLE

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INTRODUCTION: The beginning of fast motor units (MUs) tetanic contraction is characterized by highly dynamic changes in force including 200-500 ms period of very efficient initial force production (boost) followed by a subsequent force decline (reported as "sag").

AIM(S): When contractions of fast MUs are rhythmically repeated in series at short time intervals (one per second), the boost appears only in the first of them, but it may be restituted after a rest period. However, the minimum inactivity time required to restitute this phenomenon is not known and this study aimed to fill this gap.

METHOD(S): Triplets of 500 ms tetanic contractions (delivered once per second) were recorded at progressively shortening time intervals – from 90 to 2 s. The reduction of boost was assessed as a decrease of the sag amplitude after the peak force related to the boost.

RESULTS: At the beginning of recordings the boost was visible in the first contraction of successive triplets, until the time intervals become critically short. After that, as time interval shortens, boost started to diminish, and finally, completely disappeared. Moreover, the boost amplitude initially increased in parallel to the development of force potentiation. For fast resistant to fatigue (FR) MUs the estimated interval duration necessary for reduction of the sag by 50% ranged 8-29 s. For fast fatigable (FF) MUs this interval was longer as ranged 35-75 s.

CONCLUSIONS: To conclude, the boost phenomenon in unfused tetani of fast MUs is strongly dependent on activation history and type of MU. The peak force within boost is potentiating after preceding activity delivered even more than 1 minute earlier but when the intervals are shorter than ~30 s for FR and ~70 s for FF MUs the boost is reduced.

P1.14. TREADMILL TRAINING VERSUS VOLUNTARY WHEEL RUNNING: EFFECTS IN CONTRACTILE PROPERTIES OF THE THREE TYPES OF MOTOR UNITS IN RAT MEDIAL GASTROCNEMIUS MUSCLE

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INTRODUCTION: Various forms of physical activity differentially affect the neuromuscular system and have various impact on well-being of animals. Forced treadmill running (FTR), compared to voluntary wheel running (VWR), induces stress in rats as evidenced by elevated levels of corticosterone. This is particularly relevant to studies targeting brain structures sensitive to stress as hippocampus. Therefore, we have tested whether VWR could serve as a less stressful alternative form of training compared to FTR, while still eliciting beneficial adaptations.

AIM(S): This study was aimed to compare the effects of two distinct training forms (FTR and VWR) on proportion and contractile properties of motor units (MUs) in the medial gastrocnemius muscle.

METHOD(S): Male Wistar rats were assigned to 3 groups: control, FTR (4 weeks), VWR (4 weeks). Contractile properties and proportion of 3 MUs types: slow (S), fast fatigue-resistant (FR), fast fatigable (FF) were investigated during electrophysiological experiments. Additionally, running distances and medial gastrocnemius muscle mass were measured.

RESULTS: The fatigue resistance was significantly higher in FR MUs from rats subjected to FTR compared to VWR. Additionally, there were no significant differences in the proportion of three MUs types between the two training modalities.

CONCLUSIONS: The differences in fatigue resistance may be associated with the lower intensity and irregular pattern of VWR activity, which does not impose sustained FTR. Notably, both training modalities led to similar shifts in the proportions of S, FR and FF MUs types, indicating that muscle composition changes in a comparable manner regardless of whether the training is voluntary or forced. These results suggest that voluntary exercise may induce comparable neuromuscular adaptations while being less stressful. Thus, VWR could be considered as viable and less stressing alternative to traditional forced training protocols in experimental models.

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P1.15. POST-STROKE SOMATOSENSORY DEFICITS ARE REFLECTED IN ALTERED ACTIVITY OF SPINAL WDR NEURONS

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INTRODUCTION: Ischemic stroke is one of the leading causes of long-term disability. While motor deficits are widely discussed, somatosensory disorders – affecting up to 80% of patients – are often neglected. These include dysfunction in tactile sensation, proprioception, and stereognosis, significantly impairing motor recovery. Despite their prevalence, mechanisms underlying post-stroke sensory deficiencies and their dynamics remain poorly explored. Wide dynamic range (WDR) neurons in the spinal cord may reflect broader top-down changes in sensory processing after stroke.

AIM(S): To explore how ischemic stroke affects spinal WDR neurons activity over time in response to various sensory stimuli.

METHOD(S): Stroke was induced in male Sprague-Dawley rats using the Middle Cerebral Artery Occlusion (MCAO) model. The von Frey and Hargreaves behavioral tests assessed mechanical and thermal sensitivity over time. Electrophysiological recordings of lumbar WDR neurons were performed at 24h and 7 days post-MCAO using mechanical, thermal, and electrical stimuli. Infarct volume was analyzed using TTC staining and LASCA imaging.

RESULTS: We observed post-MCAO significant infarction. Behaviorally, rats showed hypoesthesia at 24h, followed by hyperesthesia by day 21 for both thermal and mechanical stimuli. Electrophysiological data showed reduced WDR responses to Aβ fiber input at 24h and enhanced responses to Aδ fibers and mechanical stimuli by day 7. Temporal summation and post-discharge were elevated at 7 days, indicating central sensitization. Additionally, diffuse noxious inhibitory control (DNIC) was absent at 24h and partially recovered by day 7.

CONCLUSIONS: Ischemic stroke leads to time-dependent disturbances in somatosensory processing, evolving from hypoesthesia to hyperesthesia and allodynia. These are paralleled by dynamic changes in WDR neuron excitability and DNIC dysfunction, suggesting impaired descending modulation. The findings emphasize the importance of spinal mechanisms in post-stroke sensory deficits.

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P1.16. PROTEOMIC ALTERATIONS AFTER SPINALIZATION: PERSPECTIVES FROM MOTONEURONS, DORSAL ROOT GANGLIA, AND EPENDYMA

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INTRODUCTION: Motoneurons (MNs) innervating soleus (SOL), a slow-twitch postural muscle, and tibialis anterior (TA), a fast-twitch phasic muscle exhibit distinct physiological and molecular features, influencing their response to spinal cord injury (SCI). Although type-specific MN responses to SCI have been reported, direct comparative proteomic data are lacking.

AIM(S): We aim to investigate proteomic differences between SOL and TA MNs in healthy rats and after spinal cord transection (SCT). We also profiled dorsal root ganglia (DRG), providing sensory input, and ependymal cells (EC) lining the central canal, supporting spinal cord homeostasis.

METHOD(S): Adult male Wistar rats (n=19) received retrograde tracers into SOL and TA. Rats were sacrificed at 2, or 6 weeks after thoracic SCT; healthy rats served as controls. Labeled SOL/ TA MNs and EC were isolated by laser microdissection (Leica). Proteins were extracted, digested, and labeled with tandem mass tags (TMT). Peptides were separated on an UltiMate 3000 nano-LC system coupled to Q Exactive HF-X mass spectrometer. Data were processed in MaxQuant (v1.6.17.0) using Andromeda search engine against the Rattus norvegicus UniProt database.

RESULTS: We identified ~1200 proteins in MNs, 1500 in EC, and 3000- 5000 in DRGs. In controls, SOL MNs exhibited higher than TA MNs expression of proteins linked to cellular homeostasis and sustained activity: PICALM (autophagy and endocytosis), CST3 (neuroprotection), LNPK (endoplasmic reticulum structure),

HAGH (metabolic detoxification), and CKAP4 (cytoskeleton-ER interaction). After SCT, MN subtypes showed distinct proteomic changes. In SOL MNs, 35 proteins changed at 2 weeks and 15 at 6 weeks, while TA MNs showed fewer changes (11 and 4, respectively). EC changes were more prominent at 2 weeks, whereas DRG sensory neuron proteomes showed greater alterations at 6 weeks.

CONCLUSIONS: Our results show both baseline and injury-induced differences between MN subtypes, emphasizing the need for precision approaches in SCI therapies.

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P1.17. IDENTIFICATION OF A NOVEL GENETIC MOUSE MODEL OF BIPOLAR DISORDER REVEALING SEX DIFFERENCES

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INTRODUCTION: Bipolar disorder is a complex neuropsychiatric condition characterized by alternating manic and depressive episodes. While existing animal models capture certain mania-like symptoms, the precise mechanisms underlying the disorder remain poorly understood.

AIM(S): Developing more accurate models is therefore essential for advancing our understanding of its pathophysiology.

METHOD(S): In this study, using multilevel approaches from molecular to behavioral characterization, we introduce a novel genetic mouse model of bipolar disorder.

RESULTS: Male knockout (KO) mice exhibited hallmark features of mania, including locomotor hyperactivity, increased risk-taking behavior, heightened sensitivity to low-dose d-amphetamine, excessive circling and turning, and episodes of backward walking. These behavioral abnormalities were consistently observed in two complementary neuron-specific conditional KO lines. Additionally, KO mice displayed dysregulated dopamine and serotonin levels across multiple brain regions, along with evidence of impaired glutamatergic transmission. Interestingly, female KO mice did not exhibit the same behavioral abnormalities as males. Instead, they showed increased anxiety-like behaviors and passive coping strategies, suggesting a sex-dependent divergence in phenotype, where males primarily exhibit mania-like traits while females display depressive-like features.

CONCLUSIONS: By closely recapitulating key aspects of bipolar disorder —including its sex-specific manifestations— this novel model represents a valuable tool for investigating the neurobiological mechanisms underlying the disorder. Furthermore, it offers a robust platform for testing potential therapeutic interventions.

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P1.18. MITOCHONDRIAL CALCIUM UNIPORTER PROTECTS HIPPOCAMPAL CA2 NEURONS FROM EXCITOTOXIC INJURY

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INTRODUCTION: The hippocampal region CA2 is exceptionally resistant to excitotoxic injury, unlike the adjacent, highly vulnerable CA1 area. Although this phenomenon is well documented, the molecular and cellular basis for such selective neuroprotection remains unclear. As calcium homeostasis differ between CA regions, and mitochondria are critical for calcium fluxes in neuronal stress, the between-region differences in mitochondrial calcium uptake may contribute to regional differences in vulnerability.

AIM(S): We aimed to determine whether the Mitochondrial Calcium Uniporter (MCU), recently identified as enriched in CA2 neurons, contributes to resistance against excitotoxicity.

METHOD(S): MCU distribution was examined using immunohistochemistry in rodent hippocampal sections. N-methyl-D-aspartate (NMDA)-exposed organotypic hippocampal slices were used as in vitro model of excitotoxicity. Region-specific changes in MCU expression and neuronal survival were studied in NMDA-exposed slices. MCU was pharmacologically inhibited using MCU-i4.

RESULTS: MCU expression was markedly higher in CA2 than in CA1, closely matching regional resistance to excitotoxicity. NMDA exposure increased MCU levels specifically in CA2. MCU inhibition sensitized intrinsically resistant CA2 neurons to NMDA-induced damage in a dose-dependent manner, while CA1 neurons, with low MCU expression, were only minimally affected.

CONCLUSIONS: Our findings show that CA2 neurons are protected against excitotoxic injury by MCU, suggesting a unique mitochondrial calcium handling in these cells. This finding highlights a context-dependent role of MCU in neuronal survival and reveals mechanisms underlying CA2's intrinsic resistance to excitotoxic stress.

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P1.19. LOST ENZYME, LOST INTEGRITY: ARGINASE 2 DELETION DISRUPTS MITOCHONDRIA IN STRIATAL NEURONS

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INTRODUCTION: Arginase 2 (Arg2) is the major cerebral arginase isoenzyme highly enriched in the striatum, a brain region critical for motor control and selectively affected in Huntingtons' disease (HD). Although Arg2 loss precedes symptom onset in HD mouse models, its role in striatal pathology remains unclear.

AIM(S): This study aimed to determine cellular localization of striatal Arg2 and assess the consequences of its loss to define its potential involvement in HD pathogenesis.

METHOD(S): Immunohistochemistry was used to identify Arg2-expressing cells. A deep learning-based segmentation and quantification tool (Cellpose), enabled precise unbiased image analysis. The effects of Arg2 loss on the striatum were investigated using Arg2 knockout (Arg2 /) mice. High-resolution NMR spectroscopy was used to compare striatal metabolite profiles in control and Arg2 / mice. Proteomic differences were assessed with LC-MS, and the integrity of electron transport chain (ETC) complexes was analyzed using blue native (BN) gel electrophoresis. Neuronal mitochondria ultrastructure was examined by electron microscopy (EM).

RESULTS: Arg2 was found to be selectively localized in medium spiny neurons (MSNs), the major projecting neurons of the striatum and primary target in HD. NMR revealed distinct metabolic profiles between control and Arg2-/- mice. Proteomic profiling revealed reduced levels of mitochondrial ETC subunits and BN gels showed decreased assembly of complexes I, III and IV. EM showed mitochondria swelling, cristae disruption, and increased fragmentation Arg2-/- neurons.

CONCLUSIONS: These findings emphasize a crucial role for Arg2 in maintaining striatal metabolism and mitochondrial integrity in MSNs. Given the selective expression of Arg2 in this neuronal population and its early loss in HD models, Arg2 may contribute to the selective vulnerability of MSNs in HD.

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P1.20. SEX-SPECIFIC ALTERATIONS IN MITOCHONDRIAL DYNAMICS AND AMPK SIGNALLING IN A TRAP1 MUTANT MICE – A NOVEL MODEL OF ASD

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INTRODUCTION: Neuronal cells depend on mitochondrial activity to maintain membrane excitability, neurotransmission, and synaptic plasticity. The AMP-activated protein kinase (AMPK) signalling pathway plays a crucial role in regulating cellular energy homeostasis and has been found to influence mitochondrial dynamics. The dynamics of these organelles determine their morphology, allowing them to adapt to metabolic requirements. Furthermore, the phosphorylation of DRP1, the major protein involved in mitochondrial fission/fusion, is essential for controlling mitochondrial dynamics, synapse maturation, synaptic transmission and plasticity. A mutation (p.Q639*) in the TRAP1 gene, which encodes the mitochondrial chaperone, was identified in an ASD patient, their monozygotic twin brother was unaffected. TRAP1 belongs to the HSP-90 family of proteins, which are involved in protecting against oxidative stress and regulating the metabolism of cells. Indeed, many neurodevelopmental disorders have been observed to be linked to mitochondrial dysfunction.

AIM(S): We investigated mitochondrial dynamics and AMPK signalling in the hippocampi of Trap1 mice

METHOD(S): Mitochondria from the hippocampi of Trap1 mutant and WT mice were isolated, and the levels and phosphorylation of proteins involved in fission and fusion were assessed in both sexes. Also, AMPK signalling was analysed, which is connected with the regulation of mitochondrial dynamics.

RESULTS: We observe sex-specific alterations in mitochondrial dynamics in the hippocampus.

CONCLUSIONS: In Trap1 mutant mice, there is a change in fission-fusion proteins level and their activity in mitochondria isolated from the hippocampus. Also, we observe differences in the regulation of mitochondrial dynamics between sexes

FINANCIAL SUPPORT: This work was supported by the National Science Centre, Poland under research projects: OPUS 2019/35/b/NZ4/04355 and OPUS 2023/51/B/NZ4/00856.

P1.21. ALTERED MITOCHONDRIAL METABOLISM IN THE BRAINS OF TRAP1 MUTANT MICE, A MODEL FOR AUTISM SPECTRUM DISORDER (ASD)

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INTRODUCTION: The brain represents the largest source of energy consumption in our body, most of the energy being primarily utilized at the synapses. Therefore, regulation of metabolite supply and energy metabolism is especially critical to the central nervous system and even subtle changes in energy production may lead to neurological diseases. Indeed, mitochondrial dysfunction was observed in a number of neurodevelopmental disorders. In an ASD patient whose identical twin was unaffected, we identified a postzygotic mosaic mutation p.Q639* in the TRAP1 gene, which encodes a mitochondrial chaperone of the HSP90 family. Additional screening of 176 unrelated ASD probands revealed an identical TRAP1 variant in a male patient who had inherited it from a healthy mother.

AIM(S): We generated knock-in Trap1 p.Q641* mice that revealed male-specific social behavior abnormalities accompanied by altered synaptic transmission and dendritic spine morphology. Next, we aimed at investigating mitochondrial metabolism in the synapses of Trap1 mutant mice.

METHOD(S): We performed functional mitochondrial phenotyping using Mitoplates (Biolog). Next, targeted metabolomics was performed to assess levels of amino acids in the brain. Finally, the levels of NAD/NADH were assessed using colorimetric assays.

RESULTS: The functional mitochondrial phenotyping of synaptoneurosomes isolated from mouse brains (cortex and hippocampus) of male and female Trap1 mice revealed differences in the use of the tricarboxylic acid cycle substrates in males but not in females. Morover, our preliminary data suggests that the levels glutamate and GABA are decreased in the hippocampus of male mutant mice, but not in females. Finally, the NAD/NADH ratio was icreased in the hippocampi of Trap1 mutant male, but not female mice.

CONCLUSIONS: Altogether, our results support the previously observed link between dysregulated mitochondrial metabolism and ASD. Moreover, our findings highlight the need for in-depth analysis of both males and females in mouse models of ASD.

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P1.22. ELECTROPHYSIOLOGICAL AND ANATOMICAL CHARACTERISTICS OF VENTRAL DENTATE GYRUS INTERNEURONS IN A RAT MODEL OF AUTISM SPECTRUM DISORDER

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INTRODUCTION: Autism spectrum disorder (ASD) is a neurodevelopmental condition associated with social deficits, anxiety, and altered hippocampal processing. The dentate gyrus (DG), particularly its ventral region (vDG), is implicated in social memory and pattern separation. Dysregulation of vDG GABAergic interneurons, especially parvalbumin (PV+) and somatostatin (SST+) cells, may underlie functional alterations observed in ASD. These interneuron populations are key regulators of granule cell excitability and neurodevelopment.

AIM(S): Therefore, the aim of this study was to investigate the electrophysiological and anatomical characteristics of vDG interneurons in a rat model of ASD.

METHOD(S): Therefore, we investigated the anatomical and electrophysiological features of the vDG neurons in male and female Sprague-Dawley rats using control animals and a valproic acid (VPA; 500 mg/kg, E12.5)-induced rat model of ASD. Immunohistochemistry was used to assess PV+ and SST+ cell density in vDG. Whole-cell patch-clamp recordings from granule cells were conducted in hippocampal slices to assess intrinsic membrane properties and inhibitory synaptic transmission.

RESULTS: Our preliminary results indicate that VPA exposure specifically affects granule cells excitability, evidenced by increased neuronal gain, while basic membrane properties and inhibitory post-synaptic currents remained unchanged. Importantly, we demonstrated that the observed effect was not depended on the number of PV+ or SST+ interneurons.

CONCLUSIONS: These findings suggest specific alternation in the intrinsic vDG granule cell excitability, without corresponding changes in GABAergic neurotransmission in ASD. Ongoing studies aim to elucidate the underlying neuronal mechanism of these effects

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P1.23. CHANGES IN BRAIN BIOELECTRICAL ACTIVITY FOLLOWING TRANSCRANIAL DIRECT CURRENT STIMULATION IN INDIVIDUALS WITH ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD) AND AUTISM SPECTRUM DISORDER (ASD)

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INTRODUCTION: Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulation technique that has attracted growing interest as a potential intervention for neurodevelopmental disorders such as Attention-Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD). Both conditions are associated with altered cortical excitability and disrupted executive functioning, making them potential targets for non-invasive neuromodulation.

AIM(S): This study aims to evaluate the effectiveness of tDCS in improving cognitive and behavioral outcomes in ADHD and ASD.

METHOD(S): A systematic review of randomized controlled trials (RCTs) published between 2015 and 2025 was conducted using PubMed, Embase, Cochrane Library, and Web of Science. A total of 320 studies were identified; 40 high-quality RCTs met the inclusion criteria and were analyzed in details.

RESULTS: Most studies reported positive effects of tDCS on working memory, attention, and inhibitory control in individuals with ADHD, and reduced stereotypical behaviors and improved communication in ASD. However, significant heterogeneity in stimulation protocols (e.g., current intensity, number of sessions, electrode size and placement) limited cross-study comparability. Long-term benefits were observed primarily in protocols involving repeated stimulation. Only a minority of studies incorporated EEG or fMRI data to link behavioral outcomes with physiological changes.

CONCLUSIONS: tDCS shows promise as a supportive intervention for ADHD and ASD. However, the lack of protocol standardization and interindividual variability limit the generalizability of findings. Personalized stimulation protocols based on neurophysiological markers may enhance treatment effectiveness.

FINANCIAL SUPPORT: This work was funded by Industry PhD Grant – Martyna Skuła (DWD/8/0125/2024) from Polish Ministry of Science and Higher Education.

P1.24. SPNAR9810Q MICE: DEEP BEHAVIORAL AND MOLECULAR PHENOTYPING OF SPONTANEOUS SPECTRINOPATHY MODEL

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INTRODUCTION: Progressive neuronal loss or proteostasis disruption underlying eurodegenerative disorders are often linked to dysregulated calpain activity. A subtype of these conditions are spectrinopathies – disorders associated with hereditary cerebellar ataxias – caused by mutations in SPTAN1 (non-erythrocytic α-II-spectrin). In previous studies, we discovered a spontaneous missense mutation in SPTAN1 gene (Sptan1 c.3293G > A: p.R1098Q) causing premature neurodegeneration in affected individuals.

AIM(S): We hypothesized that expressing human calpastatin, an endogenous calpain inhibitor, can mitigate molecular and behavioral alterations associated with the mutation.

METHOD(S): To test the hypothesis, we generated a transgenic line carrying both, the SPTAN1 mutation and human calpastatin gene (Spna2R1098Q x hCAST line). To profile gene expression in affected cerebellar tissue, total RNA was isolated and subjected to Next-Generation Sequencing, followed by differential expression profiling. Functional enrichment (e.g. GO, KEGG) and protein-protein interactions analysis were performed to identify affected biological pathways and prioritize candidate genes for further investigation. To detect subtle, mutation-driven phenotypic differences throughout developmental stages, we apply automated mice tracking in semi-naturalistic conditions using open-source tools for animal tracking (SLEAP.ai) and behavior interpretation (DeepOF). Cognitive performance are evaluated via Novel Object Recognition Test (EthoVision XT15, Noldus).

RESULTS: Our findings provide pioneer transcriptomic profile of the Spna2R9810Q mouse model. Although, we did not detect statistically significant impact of co-expressed hCAST on transcriptomic profile, 8 gene candidates were targeted for further investigation of their potential involvement in pathogenesis of SPTAN1 ataxia.

CONCLUSIONS: Ongoing behavioral studies aim to explore whether SPTAN1 mutation affects not only motor abilities, but also social behavior – an aspect not yet explored in this model.

FINANCIAL SUPPORT: This project is a part of Industrial PhD Programme: VI Edition, Ministry of Science and Higher Education, Poland (grant nb: DWD/6/00191/2022) and a part of National Science Centre grant, based on decision no. Dec-2021/41/B/NZ3/04099, entitled "Do astrocytes control synaptic connections in neural networks relevant to psychiatric diseases?".

P1.25. MOLECULAR CHALLENGES IN HEREDITARY SPINOCEREBELLAR DEGENERATIONS

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INTRODUCTION: Hereditary spinocerebellar degenerations are neurodegenerative disorders affecting mainly the spinocerebellar tracts. They include hereditary cerebellar ataxias, spastic ataxias, and spastic paraplegias. They are caused by pathogenic genetic variants in hundreds of genes.

AIM(S): Our research aims to identify new genes and causal variants, as well as genetic factors modifying clinical variability. This work describes the clinical features and molecular causes of spinocerebellar degenerations in our cohort.

METHOD(S): Our cohort from Rare Disease Reference Center for Genetic Diseases of the Nervous System at Pitié Salpêtrière Hospital and Paris Brain Institute includes 3665 families with hereditary spinocerebellar degenerations (SPATAX and BIOMOV studies, NCT00140829 and NCT05034172 respectively). For one thousand of these families, we conducted large-scale genomic explorations (exome/genome sequencing).

RESULTS: We identified a pathogenic variant in a known gene in only half of the cases. Additionally, these diseases are characterized by high clinical variability, including age at onset (from birth to late age) and and severity (from mild symptoms to bedridden). In each mode of inheritance and clinical form, there are a few main causal genes and many rare ones. Heterozygous CAG repeats expansions encoding polyglutamines in ATXN1,2,3,7 are the most frequent causes of autosomal dominant cerebellar ataxia, along with non-coding GAA expansion in FGF14. Bi-allelic non-coding GAA ex-

pansions in FXN are 58% of the known causes of autosomal recessive cerebellar ataxia. Then, SPG7, SACS, RFC1 and SETX are the other main genes in recessive forms.

CONCLUSIONS: The numerous molecular alterations and clinical heterogeneity present both challenges and clues for understanding the physiopathology of spinocerebellar ataxias. Data collection from large cohorts promotes collaborative studies and facilitates dialog between fundamental and clinical sciences.

FINANCIAL SUPPORT: APHP, Inserm, Paris Brain Institute, Fondation Médicale pour la Recherche (FRM).

P1.26. INTER-STRAIN CHIMERAS REVEAL NEURODEVELOPMENTAL MECHANISMS OF CORPUS CALLOSUM AGENESIS IN A MOUSE MODEL

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INTRODUCTION: The corpus callosum (CC) is the major axon tract of the eutherian brain, connecting and integrating inter-hemispheric neural activities. Developmental defects leading to CC dysgenesis or agenesis affect 1: 4000 children worldwide, however, their etiology and pathogenesis remain poorly understood.

AIM(S): To get insights into developmental mechanisms, here, we investigated CC development in inter-strain chimeras generated by combining stem cells and embryos of the BTBR T+ Itpr3tf/J (BTBR) mouse model of CC agenesis with those of the C57BL6/J (B6) control strain.

METHOD(S): BTBR and B6 embryonic stem cells (ESCs) were first engineered to express a TAU-GFP transgene, and subsequently injected into preimplantation embryos, which were then transferred to female recipients to generate chimeric mice. This approach allowed tracing axonal projections, including the CC, in the brains of BTBR<->B6 chimeras.

RESULTS: We found that ESCs of either strain engraft into the host embryo of the other strain and contribute stochastically to multiple brain areas, neuronal circuits, and non-neuronal brain tissues. The CC was present in the brain of 80% of chimeras obtained from BTBR ESCs engrafted into B6 host, and in 15% of chimeras obtained from B6 ESCs engrafted into BTBR host. Remarkably, we found that BTBR ESC-derived cortical neurons can develop callosal inter-hemispheric axonal projections when engrafted in a B6 host embryo.

CONCLUSIONS: These results highlight the intrinsic ability of BTBR neurons to develop callosal inter-hemispheric projections and suggest the involvement of early-life interactions between callosal neurons and non-neuronal cells in determining midline crossing and CC formation. Overall, this study supports the use of inter-strain chimeric mice to unveil mechanisms underlying brain developmental defects, such as CC agenesis.

FINANCIAL SUPPORT: This research was supported by the National Science Center, Poland (grant no. 2020/39/B/NZ4/02105).

P1.27. BEYOND CEREBRUM AND NEUROSURGERY: A SYSTEMATIC REVIEW ON CHIARI MALFORMATION TYPE I INTEGRATING MOLECULAR AND BEHAVIORAL METHODOLOGIES

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INTRODUCTION: Chiari malformation type 1 (CM1) radiologically defined as a caudal displacement of cerebellar tonsils exceeding 5 mm through the foramen magnum, represents the most prevalent subtype of Chiari Malformations—a group of developmental disorders characterized by congenital posterior fossa deformities. Given CM1's predominantly asymptomatic or paucisymptomatic clinical course, epidemiological data primarily reflects symptomatic cases, with prevalence traditionally estimated between 0.1% and 1%.

AIM(S): We endeavor to propose a transdisciplinary conceptualization of CM1 that illuminates the cerebel-

lum's extensive non-motor functions.this comprehensive review synthesizes evidence from over 60 original research studies spanning genetics, genomics, neuropsychology, and neuropsychiatry.

METHOD(S): Our analysis deconstructs the genetic, familial, and environmental factors through a framework of "CM1 Equifinality" in disease pathogenesis, methodically investigating its significant co-occurrence with neurodevelopmental and psychiatric conditions—particularly autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) in both syndromic and isolated cases, where behavioral and genomic tools are consistently validating each other,

RESULTS: CM1 frequently presenting alongside functional impairments and problems with self-regulation, as further documented through combined measures of neuropsychology and functional neuroimaging.

CONCLUSIONS: With this work, we want to emphasize the critical need for psychiatric monitoring, neuropsychological interventions, and psychotraumatological implications in high-risk pediatric populations for CM1 within the general population. We also want to highlight how CM1 represents a unique model of study integrating developmental biology, cognition, and psychiatry through a cerebellar lens. We propose CblOs as a potential tool foreseeing the use of 3D in vitro models to recapitulate the cellular and molecular features of ASD and ADHD in CM1.

FINANCIAL SUPPORT: No.

P1.28. TARGETING NEUROINFLAMMATION IN THE BRAIN: EFFECTS OF THE PUTATIVE CANNABINOID RECEPTOR GPR55 LIGANDS CBD, O-1602, AND ML-193 IN MIXED GLIAL CELL CULTURES

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INTRODUCTION: Astrocytes and microglia are the primary resident glial cells involved in the initiation, propagation, and resolution of neuroinflammation. Cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) play pivotal roles in the pathophysiology of neurodegenerative disorders, including Alzheimer's disease and neuropathic pain. GPR55, a G-protein-coupled receptor, modulates inflammatory responses and is a putative cannabinoid receptor, suggesting its involvement in regulating neuroinflammation.

AIM(S): This study aimed to investigate the effects of GPR55 ligands: CBD, ML-193, and O-1602 on cortical astrocytes and microglia in primary mixed glial cultures, with and without IL-1β+TNF-α cotreatment.

METHOD(S): Immunocytochemistry was used to verify GPR55 expression in microglia and astrocytes, and to assess the compounds' effects on markers including SOX2, Iba-1, CD11b, BDNF, GFAP, Nestin, and Ki-67. Morphological analyses of Iba-1-positive cells were performed (area and perimeter). IL-6 concentrations in culture media were quantified using ELISA.

RESULTS: GPR55 expression was confirmed in both astrocytes and microglia. ML-193 cotreatment with IL-1β + TNF-α significantly increased IL-6 levels in a dose-dependent manner. No significant IL-6 changes were observed following CBD or O-1602 treatment. ML-193 treatment also reduced microglial cell numbers and induced morphological alterations under both basal and inflammatory conditions.

CONCLUSIONS: ML-193 exhibits pro-inflammatory effects in mixed glial cultures, promoting IL-6 release and microglial cell loss. These findings support a modulatory role of GPR55 in neuroinflammation and suggest ML-193 as a potential pro-inflammatory agent in glial contexts.

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P1.29. B-HYDROXYBUTYRATE ALTERS NEUROINFLAMMATORY RESPONSE AND CELL MIGRATION ABILITIES AFTER THE SCRATCH INJURY IN VITRO

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INTRODUCTION: The ketogenic diet, a high-fat, low-carbohydrate regimen, has gained attention for its neuroprotective potential in neurological disorders. β-Hydroxybutyrate (BHB), a ketone body, serves as an alternative energy substrate and modulates inflammation.

AIM(S): This study aimed to evaluate the impact of BHB on the cellular response to scratch injury in rat cortical and hippocampal primary cultures.

METHOD(S): The cortices and hippocampi were isolated from the brains of rat pups, and mixed neuronal-glial cultures were established. On days 14–21 in vitro (DIV), the medium was supplemented with 5 mM BHB. On DIV21, a scratch wound was introduced using a pipette tip to simulate brain injury. Cell migration was monitored using the CytoSMART™ Lux2 Live Cell Imager for 24 hours. The expression of GFAP and NeuN (astrocytic and neuronal markers) was assessed via Western blot in cell lysates at 6 and 24 hours post-injury. The expression of inflammatory markers was assessed using antibody arrays 6 hours post-injury. At 24 hours post-injury, cells were fixed and prepared for electron microscopy.

RESULTS: BHB treatment prior to injury reduced migration speed in cortical cultures and altered the inflammatory response—reducing the expression of IL-1β while increasing levels of IFNγ and granulocyte-macrophage colony-stimulating factor (GM-CSF). In hippocampal cultures, BHB had no effect on migration speed but increased the expression of neuropilin-1. Moreover, changes in GFAP expression were observed in BHB-supplemented hippocampal cultures—a decrease in GFAP levels 6 hours post-injury compared to non-injured controls, followed by an increase at 24 hours post-injury. Ultrastructural analysis revealed a large number of lipid droplets and active lipophagy in BHB-treated cells.

CONCLUSIONS: In summary, BHB alters the cellular response to injury and may have region-specific effects on brain cells.

FINANCIAL SUPPORT: Funding: National Science Centre Preludium21 Grant 2022/45/N/NZ4/03028.

P1.30. OXIDATIVE STRESS IN PRIMARY MICROGLIAL CELLS DUE TO EXPOSURE TO PLASTIC NANOPARTICLES

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INTRODUCTION: Plastic is a material with a wide range of applications across various industries, with polystyrene (PS) being most popular in commercial sectors. Its widespread use contributes to the increased occurrence of micro- (MP) and nanoparticles (NPs) in the environment, which raises concerns about their harmful impact on living organisms. Their prevalence creates a risk of increased exposure and raises questions about their long-term effects, especially on the central nervous system after crossing the blood-brain barrier.

AIM(S): Therefore, we determined the effect of PS-NPs on primary microglial cells in vitro, with particular emphasis on oxidative stress, which underlies many neurological disorders, including neurodegenerative diseases.

METHOD(S): Commercially purchased PS-NPs were suspended in culture medium and administered at concentrations of 1, 25 and 50 μg/mL for 24-48 h to primary microglial cells that were collected from the brain of Wistar pups. Fluorescently labelled PS-NPs were visualized after staining the cells with the microglial marker (IBA-1) and the images were assessed by confocal microscopy. Reagent kits were used to analyze cell viability (MTT, LDH) and evaluate the levels of oxidative stress markers.

RESULTS: Exposure to PS-NPs led to decreased viability of primary microglial cultures in time- and concentration depended manner. The cells were characterized by morphological changes caused by excessive phagocytosis-dependent intracellular accumulation of particles. The presence of oxidative stress markers, such as increased production of reactive oxygen species (ROS), was observed.

CONCLUSIONS: Overproduction of ROS due to oxidative stress can induce irreversible changes in microglial proteins and lipids. Moreover, constant exposure to plastic NPs can lead to the development of neuroinflammation, potentially contributing to neurological disorders.

FINANCIAL SUPPORT: The study was financed by Mossakowski Medical Research Institute, Polish Academy of Sciences (Warsaw, Poland) grant no FBW-07/2024.

P1.31. THERAPEUTIC POTENTIAL OF PAPE-1 AGAINST HYPOXIC/ISCHEMIC BRAIN INJURY: A DUAL NEURON – GLIA TARGETED APPROACH

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INTRODUCTION: Hypoxic-ischemic brain injuries are among the leading causes of mortality and long-term disability worldwide. Despite ongoing advances in neuroscience and clinical medicine, the development of effective therapeutic interventions for these conditions remains a major challenge.

AIM(S): In this context, we propose the application of Pathway Preferential Estrogen-1 (PaPE-1), a novel synthetic compound that selectively activates non-nuclear estrogen receptors. This mechanism enables rapid, non-genomic cellular responses while reducing the risk of side effects commonly linked to classical estrogen receptor activation.

METHOD(S): Our research integrates in vitro models employing primary mouse cortical neurons and human microglial cells subjected to hypoxic/ischemic conditions, followed by post-treatment with PaPE-1 to reflect clinically relevant therapeutic paradigms. Simultaneously, we are developing the photothrombotic stroke model as a preliminary in vivo tool to study focal cerebral ischemia and assess PaPE-1's therapeutic potential.

RESULTS: Our original findings indicate that PaPE-1 exhibited pronounced neuroprotective efficacy in hypoxic/ischemic models through the modulation of key pathological pathways, including apoptosis, autophagy, and oxidative stress. Treatment with PaPE-1 restored the expression of neuronal injury biomarkers, indicative of preserved cellular integrity and functionality. Furthermore, PaPE-1 regulated neuroimmune signaling in both primary neuronal and human microglial cultures, underscoring its comprehensive mechanism of action and potential as a therapeutic agent for ischemic brain injury.

CONCLUSIONS: Based on these findings, we postulate that post-treatment with PaPE-1 exerts a multifaceted modulatory effect on diverse cellular pathways involved in hypoxic/ischemic injury. This cumulative influence underscores the potential of PaPE-1 as a promising therapeutic agent for neuroprotection, capable of mitigating neuronal damage and promoting recovery following hypoxic/ischemic insults.

FINANCIAL SUPPORT: The National Science Centre, Poland: grant no. 2021/43/D/NZ7/00633.

P1.32. SORCS2 SHIELDS ASTROCYTES FROM AMYLOID BETA (AB) STRESS BY REGULATING P75 NTR SIGNALING

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INTRODUCTION: Alzheimer's disease (AD) is the leading cause of dementia, marked by amyloid-beta (Aβ) accumulation, inflammation, and neurodegeneration. Astrocyte dysfunction impacts lipid metabolism and proteostasis, contributing to AD progression. SorCS2 protects astrocytes under Aβ exposure, but its exact role remains unclear. The p75NTR contributes to apoptosis, with its activity being regulated by lipid homeostasis and S-palmitoylation, as well as influenced by membrane dynamics. A promising therapeutic molecule, LM11A-31, has been shown to downregulate p75NTR, leading to cognitive improvements in AD patients. However, the complex interplay between SorCS2, p75NTR, and lipid metabolism in astrocyte survival requires further investigation.

AIM(S): The study investigated SorCS2's involvement in lipid homeostasis and p75NTR-mediated apoptosis in astrocytes exposed to Aβ.

METHOD(S): Primary astrocytes from wild-type (WT) and SorCS2-knockout (KO) mice were exposed to

Aβ. Fluorescent lipid assays were performed to quantify cholesterol levels, microscopy-based analysis was conducted to examine lipid droplet formation, and Western blot and immunostaining were used to assess S-palmitoylation.

RESULTS: SorCS2 deficiency disrupts lipid metabolism and impairs lipid droplet accumulation in astrocytes. Elevated p75NTR protein levels were observed, accompanied by transcriptional upregulation. Preliminary data indicate that lipid abnormalities may induce aberrant S-palmitoylation, affecting the subcellular distribution of p75NTR, TRAF6, and p62 proteins involved in neurotrophin signaling and stress responses. Additionally, early observations suggest changes in plasma membrane lipid composition, potentially impacting membrane fluidity and trafficking dynamics.

CONCLUSIONS: This study clarifies SorCS2's role in astrocyte survival, shedding light on lipid-driven astrocytic dysfunction and its implications for AD therapy

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P1.33. SORCS2 SHAPES THE SECRETOME OF ASTROCYTES ACTIVATED AFTER ISCHEMIC STROKE

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INTRODUCTION: Ischemic stroke, caused by obstructed cerebral blood flow, results in neuronal death and astrocyte activation (astrogliosis). While current treatments focus on restoring circulation, increasing attention is directed toward the role of astrocytes in post-stroke brain repair, particularly in inflammation, glial scar formation, and extracellular matrix (ECM) remodeling. Transforming growth factor-beta (TGFβ), an immunomodulatory cytokine upregulated after stroke, potently activates astrocytes and modulates their secretory profile. Among the factors induced by TGFβ in astrocytes is SorCS2, a VPS10P domain receptor involved in intracellular protein sorting.

AIM(S): This study aims to investigate the role of SorCS2 in the TGFβ-dependent secretory activities of astrocytes, with a focus on ECM protein release and its implications for inflammation after ischemic stroke.

METHOD(S): We performed mass spectrometry-based analysis of secretomes from wild-type and SorCS2-knockout primary murine astrocytes treated with TGFβ. We employed the Middle Cerebral Artery Occlusion (MCAo) model in mice to assess in vivo effects of SorCS2 deficiency on ECM composition and immune cell morphology in post-stroke brain tissue.

RESULTS: Secretome profiling revealed that SorCS2-deficient astrocytes exhibited impaired secretion of key ECM molecules, including biglycan, agrin, neurocan, and thrombospondin-1 (Thbs1), in response to TGFβ. In vivo, SorCS2 knockout mice showed reduced levels of several ECM proteoglycans and displayed altered morphology of myeloid cells in the ischemic hemisphere compared to controls.

CONCLUSIONS: These findings suggest that SorCS2 is essential for ECM remodeling and inflammatory responses, providing insights into potential therapeutic targets for post-stroke therapeutic strategies.

FINANCIAL SUPPORT: This study was supported by the National Science Center, Poland 2020/37/B/NZ3/00761.

P1.34. ALTERATIONS OF IMMUNE CHECKPOINT PROTEINS IN ANIMAL MODELS OF DEPRESSION-LIKE BEHAVIOR

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INTRODUCTION: A growing body of research links depression to activation of immuno-inflammatory pathways. Although current antidepressants for depressive disorders show some immunomodulatory effects, exploring novel therapeutic targets remains a critical priority.

AIM(S): This study aimed to investigate the involvement of inhibitory immune checkpoint (ICP) molecules—namely programmed cell death protein 1 (PD-1) and its ligand PD-L1—as well as activatory ICPs, including Inducible T-cell COStimulator (ICOS) and CD28, in depression-like behavior using animal models.

METHOD(S): The study employed three animal models: WKY rats (treatment-resistant depression), Wistar rats subjected to 21-day chronic restraint stress (CRS), and C57BL/6 mice infected with Porphyromonas gingivalis (infection-induced depression). Untreated Wistar rats served as controls for both rat models. The protein levels of specific ICPs were measured in the hippocampal, cortical, and splenic tissues derived from the examined animals.

RESULTS: In WKY rats, increased PD-1 and decreased PD-L1 levels were observed in the spleen. Activating immune checkpoints (ICOS and CD28) were elevated in the hippocampus and spleen of WKY rats and CRS-exposed rats and in the hippocampus of P. gingivalis-infected mice. No significant changes were found in the frontal cortex. These results highlight model-specific alterations in immune checkpoint expression linked to depression-like behavior.

CONCLUSIONS: The observed alterations in immune checkpoint protein levels across different animal models suggest that ICP regulation may play a key role in the pathophysiology of depression-like behaviors. These findings highlight potential therapeutic targets involving immune checkpoint pathways for the treatment of depression.

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P1.35. INFLUENCE OF PSYCHOSOCIAL OVERCROWDING STRESS ON THE EXPRESSION OF INFLAMMATORY FACTORS IN THE RAT COLON

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INTRODUCTION: Chronic stress is a well-known modulator of inflammatory responses in the gastrointestinal tract, leading to increased intestinal permeability and dysregulation of the gut-brain axis. However, the mechanisms underlying the disruption of intestinal barrier integrity remain poorly understood.

AIM(S): The aim of this study was to assess the effect of 14-day overcrowding stress on the mRNA levels of pro-inflammatory (IL-1β, TNF, IL-6, MIF, PTGS2, NOS2, IFN-γ, CD163) and anti-inflammatory factors (IL-10, Tgfbr2, ARG2, IL-4) in the rat colon.

METHOD(S): Male Wistar HAN rats were divided into a control (CON) and a crowding stress (CS) group. To assess long-term effects, subsets of CS rats underwent recovery periods of 7, 14, or 21 days. On the final experimental day, distal colon segments were collected for RNA isolation, reverse transcription, and TaqMan probe-based qPCR.

RESULTS: Exposure to CS resulted in increased mRNA levels of TNF, Tgfbr2, NOS2, MIF, CD163, and ARG2. Except for TNF, the elevated levels of these molecules persisted for at least 7 days after cessation of CS exposure; in the case of NOS2 and ARG2, the increase was augmented.

CONCLUSIONS: Our results demonstrate the long-lasting impact of psychosocial stress on intestinal homeostasis. These findings provide new evidence for the link between psychological stress and immunological disturbances in the gut, supporting the brain-gut axis as a key mediator of these interactions.

FINANCIAL SUPPORT: This work was supported by statutory funding from the Department of Brain Biochemistry, Institute of Pharmacology, Polish Academy of Sciences.

P1.36. MODULATION OF NMDA RECEPTOR SUBUNIT EXPRESSION BY (R,S)-SULFORAPHANE IN THE OLFACTORY BULBECTOMY MODEL IN MICE

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INTRODUCTION: Dysregulation of glutamatergic transmission via NMDA receptors is increasingly recognized as a key contributor to the pathophysiology of depression . The olfactory bulbectomy (OB) is a well-established animal model that displays behavioral and molecular features characteristic of agitated depression. (R,S)-Sulforaphane (SFN) is a bioactive compound known for its antioxidant and neuroprotective properties, primarily through activation of Nrf2 pathway. Emerging evidence suggests its potential to modulate glutamatergic signaling and antidepressant-like activity . However, its influence on NMDA receptor subunit expression in depression remains unclear.

AIM(S): Investigation the effects of repeated SFN treatment on GluN2A and GluN2B expression in the frontal cortex (FCx) and hippocampus (Hp) in OB mice.

METHOD(S): Brain samples (FCx and Hp) were obtained from mice subjected to the OB procedure followed by 14-day treatment with (R,S)-sulforaphane (10 mg/kg i.p.; SFN10). Control (Sham) and reference drug (Amitriptyline 10 mg/kg i.p.; AMI10) groups were included in this study. Protein levels of GluN2A and GluN2B were determined by Western blot, and mRNA levels of Grin2a and Grin2b were assessed using Real-Time PCR. Statistical analysis was conducted with one-way ANOVA (GraphPad Prism v10.0).

RESULTS: OB procedure did not significantly change on GluN2A and GluN2B protein levels, both in the FCx and Hp. However, SFN10 treatment significantly decreased GluN2B protein levels in the FCx of OB mice ($p<0.01$), with no changes observed in the Hp. At the mRNA level, OB had no significant effect on Grin2a or Grin2b expression. Notably, both SFN10 and AMI10 (R,S) significantly reduced Grin2b mRNA levels in the FCx of OB mice ($p<0.001$). In contrast, only SFN10 induced a significant increase in Grin2b expression in the Hp ($p<0.05$). SFN 10 and AMI10 treatment did not affected Grin2a mRNA expression in either brain region.

CONCLUSIONS: These findings indicate that (R,S)-sulforaphane may in modulate NMDA receptor subunits expression in a specific brain region. Moreover, the obtained results suggest a potential molecular target involved in this regulation, specifically related to the GluN2B subunit.

FINANCIAL SUPPORT: The study was partially supported by the PRELUDIUM grant from the National Science Centre contract: UMO-2016/23/N/NZ4/01337 provided to P. Pańczyszyn-Trzewik.

P1.37. EFFECTS OF CHRONIC MILD STRESS AND ESCITALOPRAM ON GLUCAGON-LIKE PEPTIDE-1 AND FUNCTIONALLY RELATED PROTEINS IN THE FRONTAL CORTEX OF RATS

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INTRODUCTION: Depressive disorders are associated with neurobiological alterations in the brain involving both serotonergic signaling and neurotrophic factors regulating neuronal plasticity [Pannu et al.,2023;Yang et al.,2020]. Recently, gut-derived peptides such as glucagon-like peptide-1 (GLP-1) have gained attention for their potential role in brain function modulation [McIntyre et al.,2025].

AIM(S): To investigate the effects of chronic mild stress (CMS;animal model of depression) and escitalopram (ESC; a selective serotonin reuptake inhibitor) treatment on the levels of GLP-1 and functionally related proteins (GLP-1R, BDNF, TrkB) in the frontal cortex (FCx) of rats.

METHOD(S): Brain samples (FCx) were obtained from rats subjected to the CMS procedure (7 weeks;Stress NaCl) and 35-day escitalopram treatment (10 mg/kg;Stress ESC). Appropriate non-stressed control groups were included (CTR NaCl;CTR ESC) in this study. GLP-1 concentration was measured using a commercial ELISA kit. Protein levels of GLP-1R, BDNF, TrkB were assessed by Western blot analysis. Statisti-

cal analysis was performed using two-way ANOVA in GraphPad PRISM v10.0.

RESULTS: The CMS procedure significantly reduced GLP-1 concentration in rats' FCx ($p<0.05$; CTR NaCl *vs.* Stres NaCl), while ESC10 administration reversed these changes ($p<0.01$; Stres NaCl *vs.* Stress ESC). Two-way ANOVA revealed significant effect of drug ($F1,19=5.505$, $p=0.0367$), no effect of stress ($F1,19=0.04$, $p=0.8491$) and significant interaction ($F1,19=16.51$, $p=0.0006$). These changes were accompanied by reductions in GLP-1R ($p=0.004$), BDNF ($p=0.0156$), and a trend toward decreased TrkB ($p=0.0561$) in stressed animals, which were partially reversed following escitalopram administration.

CONCLUSIONS: Our findings suggest that chronic stress and escitalopram may modulate neuropeptidergic and neurotrophic signaling in the frontal cortex, with alterations in GLP-1, GLP-1R, BDNF, TrkB levels potentially linking stress pathophysiology to antidepressant action.

FINANCIAL SUPPORT: The study was partially supported by grants from the National Science Centre (contracts: UMO-2016/21/B/NZ7/01623 to M. Sowa-Kucma) and by fund of the University of Rzeszów (grant: NIW/8/2024).

P1.38. DISTINCT MICRORNA SIGNATURES OF CHILDHOOD TRAUMA IN HUMAN SERUM AND SPERM: IMPLICATIONS FOR POTENTIAL INTERGENERATIONAL TRANSMISSION

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INTRODUCTION: Childhood trauma has been associated with long-term behavioral and metabolic sequelae across generations. Emerging evidence from rodent studies supports a role for sperm RNAs in the intergenerational transmission of neuropsychiatric and metabolic disease susceptibilities through the patriline. However, the translational relevance of this concept in humans remains understudied.

AIM(S): We systematically examined small RNAs in the serum and sperm samples from different human trauma cohorts to synthesize evidence for the plausibility of intergenerational transmission of susceptibilities in humans. These include Pakistani children (n=72, n=42 controls) and adult men (n=93) with histories of complex childhood trauma, as well as Bosnian families (n=22, n=20 controls) who lived through the genocide during their formative years.

METHOD(S): Small RNA sequencing (sRNA-seq) followed by RT-qPCR assays were performed in the collected serum and sperm samples from the Pakistani and Bosnian trauma cohorts. The data was compared and correlated with the neuropsychiatric scales and lipid profiles of the subjects intergenerationally.

RESULTS: sRNA-seq revealed differential expression of 48 miRNAs in the serum of traumatized children *vs.* controls in Pakistan, whereas 29 miRNAs are altered in the sperm of trauma-exposed Pakistani men compared to controls. Similarly, a number of miRNAs were differentially expressed in the sperm of Bosnian men exposed to genocide. Importantly, neuropsychiatric symptoms in the Bosnian children correlated with miRNAs expression in the sperm of their fathers.

CONCLUSIONS: Collectively, these findings underscore the potential role of serum and sperm miRNAs in the intergenerational transmission of trauma-related phenotypes in humans and support the candidacy of certain miRNAs as biomarkers of such effects.

FINANCIAL SUPPORT: SONATA, MUSE ACE grants.

P1.39. FROM DIET TO DEMENTIA: THE WESTERN DIET'S INFLUENCE ON ALZHEIMER'S VIA THE LIVER-BRAIN AXIS AND SYSTEMIC MARKERS

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INTRODUCTION: The Western diet (WD) includes ultra-processed foods rich in simple carbohydrates, salt, saturated fats, and cholesterol, but low in whole grains, fiber, and unsaturated fatty acids. Originating in the USA, it has spread globally with lifestyle changes linked to technological advancement. Alzheimer's disease (AD) appears in early-onset familial (FAD) and late-onset sporadic (SAD) forms. FAD is caused by mutations in APP, PS1, and PS2 genes, while SAD is mainly influenced by environmental and lifestyle factors.

AIM(S): Investigate the role of the liver-brain axis in AD progression by examining the effects of WD on peripheral metabolic parameters in plasma and liver, as well as amyloid-beta (Aβ) peptide deposition in the liver, followed by progressive brain amyloidosis.

METHOD(S): Transgenic Tg2576 mice (FAD model) and wild-type C57BL/6 mice (SAD risk model) were fed WD or standard diets. Metabolic parameters in blood were assessed biochemically, and liver and brain tissue were analyzed for Aβ accumulation by immunofluorescence.

RESULTS: WD rapidly induced metabolic syndrome, including hypercholesterolemia, insulin resistance, hypoglycemia, and elevated levels of liver enzymes. Even short-term exposure caused NAFLD with hepatic fat accumulation, immune infiltration and hepatocyte damage. Liver dysfunction correlated with impaired Aβ clearance by hepatocytes, resulting in Aβ buildup in liver and brain. WD accelerated Aβ deposition in Tg2576 mice and promoted age-related Aβ accumulation in C57BL/6 mice.

CONCLUSIONS: WD, via disruption of liver function, contributes to AD pathogenesis by enhancing Aβ accumulation in the brain. These findings highlight the interplay between diet, systemic metabolism, and neurodegeneration. Peripheral blood markers may serve as early indicators for AD risk and targets for prevention.

FINANCIAL SUPPORT: This study was supported by the Polish National Science Centre, project No. 2014/15/D/NZ4/04361.

P1.40. BRAIN ENERGY IMBALANCE AND INFLAMMATORY RESPONSE IN BBB DYSFUNCTION IN APOE-/-/LDLR-/- MICE DURING HYPERCHOLESTEROLEMIA

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INTRODUCTION: Hypercholesterolemia is a key metabolic disturbance frequently associated with an increased risk of cardiovascular and neurodegenerative disorders. Growing evidence suggests that elevated cholesterol levels adversely affect the neurovascular unit (NVU), leading to blood-brain barrier (BBB) dysfunction, altered cerebral energy metabolism, and enhanced neuroinflammatory processes.

AIM(S): This study aimed to investigate how chronic hypercholesterolemia alters BBB integrity and inflammatory signaling in the brain, with a specific focus on extracellular adenine nucleotide metabolism in brain microvascular endothelial cells using a genetically modified mouse model.

METHOD(S): Three-month-old ApoE-/-/LDLR-/- double knockout mice and age-matched C57BL/6 control mice were used. The expression and activity of e-NTPDase, ecto-5'-NT, and eADA were assessed by HPLC. BBB integrity was examined using immunofluorescent detection of isothiocyanate-dextran (FD40). Levels of pro-inflammatory cytokines IL-1β and IL-6 were measured with ELISA.

RESULTS: In hypercholesterolemic mice, we observed a significant increase in eADA activity, elevated BBB permeability indicated by greater FD40 leakage, and increased concentrations of IL-1β and IL-6 in brain tissue compared to controls.

CONCLUSIONS: Hypercholesterolemia leads to impaired brain energy metabolism, BBB disruption, and a pro-inflammatory microenvironment. The observed metabolic shift toward inosine synthesis may reflect a neuroprotective and immunomodulatory response aimed at preserving endothelial cell viability.

FINANCIAL SUPPORT: This study was supported by statutory funding from the Medical University of Gdańsk.

P1.41. COMPENSATORY NEUROGENESIS AND NEUROPEPTIDE NETWORK IN THE PREVENTION OF HYPERPHAGIA IN A DICER-DEFICIENT MOUSE MODEL OF HYPOTHALAMIC OBESITY

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INTRODUCTION: MicroRNAs (miRNAs) are essential post-transcriptional regulators of gene expression, particularly in the central nervous system. The RNase III enzyme Dicer is critical for the maturation of miRNAs. Conditional deletion of the Dicer1 gene in the hypothalamus has been shown to result in hyperphagic obesity in mice, implicating miRNA pathways in the regulation of energy homeostasis. We hypothesize that the transient nature of obesity observed in hypothalamic Dicer-deficient mice is driven by compensatory neurogenesis, which restores homeostatic control through altered neuropeptidergic signaling.

AIM(S): This study aims to investigate the dynamics of neuropeptide expression in the hypothalamus following Dicer deletion and to assess whether neurogenesis contributes to the normalization of food intake and body weight.

METHOD(S): Using a Dicer CaMKCreERT2 mouse model, we induced Dicer deletion in the adult male and female mice. Mice were sacrificed at 3, 6, and 9 weeks post-induction to capture key phases of the obesity phenotype—onset, peak, and resolution. Hypothalamic tissue was collected for neuropeptidomic analysis. To assess cell proliferation, BrdU was administered systemically prior to tissue collection.

RESULTS: In accordance with prior observations, food intake and body weight reach a maximum around 5–6 weeks following mutation induction. This is followed by a spontaneous decline by weeks 8–9, with levels approaching those observed in control animals. Subsequent analyses will ascertain whether this phenotypic reversal is associated with particular neuropeptide alterations and elevated markers of neurogenesis.

CONCLUSIONS: The present study will provide insight into the molecular and cellular mechanisms underlying the transient obesity phenotype in Dicer-deficient mice. A comprehensive understanding of the role of neurogenesis and neuropeptide remodeling is essential for identifying novel therapeutic targets in the context of hypothalamic obesity.

FINANCIAL SUPPORT: OPUS.

P1.42. SEX-DEPENDENT NEURONAL ACTIVATION AND BEHAVIOURAL DYSFUNCTION CAUSED BY NTG-INDUCED MIGRAINE IS REVERSED BY NOP RECEPTOR AGONIST

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INTRODUCTION: Migraine is a debilitating neurological disorder associated not only with sensory disturbances but also with impairments in social behavior. However, the neural mechanisms underlying migraine-associated social dysfunction remain poorly understood.

AIM(S): This study aimed to identify specific brain regions contributing to migraine induced pain and social impairments and to investigate potential sex-dependent differences.

METHOD(S): Migraine-like symptoms were induced using nitroglycerin (NTG) in TRAP2/Ai9 transgenic mice. Neuronal activation was assessed in brain regions associated with pain and social behavior. Behavioral analyses included the three-chamber social novelty test and von Frey assay for mechanical allodynia. To assess therapeutic potential, the nociceptin/orphanin FQ peptide (NOP) receptor agonist Ro 64-6198 was administered.

RESULTS: NTG induced significant neuronal activation in the anterior cingulate cortex, amygdala, hippocampus, hypothalamus, and periaqueductal gray in female mice, and in the trigeminal nucleus caudalis in both sexes ($p<0.05$). Behaviorally, NTG led to social avoidance, reflected by increased time spent in the neutral chamber ($p=0.0003$). Male mice showed decreased direct contact with both familiar ($p<0.05$) and non-familiar ($p=0.0048$) mice, whereas female mice displayed reduced time in the chamber with the non-familiar mouse ($p<0.05$). NTG also induced robust mechanical allodynia. Administration of Ro 64-6198 reversed both the allodynia and social deficits ($p<0.0001$ in males; $p=0.0092$ in females) and significantly reduced NTG-induced neuronal activation ($p<0.0001$ in males; at least $p<0.05$ in females).

CONCLUSIONS: These findings highlight sex-dependent neural and behavioral alterations underlying migraine-associated social dysfunction. Brain regions implicated in both pain and social behavior mediate these effects, and NOP receptor activation represents a promising therapeutic strategy extending beyond pain relief.

FINANCIAL SUPPORT: This work was supported by the grant from the Polish National Science Center (SONATA BIS 11 funding 2021/42/E/NZ7/00191) to KT-D.

P1.43. THE EFFECT OF RETINAL DOPAMINE SIGNALING ON THE BEHAVIORAL RHYTHMS

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INTRODUCTION: Parkinson's disease is one of the most common age-related disease in the modern times. Motor symptoms observed in patients are related with the degeneration of dopaminergic cells in substantia nigra. Recent studies have shown that one of the first symptoms are those related to vision, caused by decreased dopamine signaling in the retina. The fruit fly (Drosophila melanogaster) is the good alternative for modeling Parkinson's disease. The presence of receptor Dop1R1 in the Drosophila eye has been identified, but its function in this location has not been identified.

AIM(S): The main aim of the study was to show the effect of the reduction of the Dop1R1 receptor expression in the retinal cells of Drosophila melanogaster.

METHOD(S): The shape and structure of the retina was examined to describe potential effect of decreased dopamine signaling on the ommatidia degeneration. We checked also possible changes in the sleep level, as it was shown previously that proper retina functioning is necessary to maintain the normal pattern and level of sleep and activity.

RESULTS: Our results indicate that even no changes in ommatidia degeneration were detected between the experimental and the control groups, increased locomotor activity during the dark phase was observed. There was also statistically significant difference in the time of the period of circadian activity.

CONCLUSIONS: The Dop1R1 receptor present in the eye plays a significant role in the regulation of circadian activity in the dark phase, suggesting that dopamine has a significant impact on circadian clock signaling already at the level of ommatidia. This study helps to understand the mechanism involved in dopamine signaling in the visual system of the fruit fly and it may help to understand the mechanisms of eye degeneration in parkinson's disease.

P1.44. MITOPHAGY DISRUPTION LEADS TO THE CIRCADIAN RHYTHMICITY DISORDER IN DROSOPHILA MELANOGASTER

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INTRODUCTION: Parkinson's disease (PD) is one of the most common age-related neurodegenerative disorders, connected with the loss of dopaminergic neurons in the substantia nigra. It's early-onset form is associated with mutation in the park gene. PARKIN protein initiates process of ubiquitination and degradation of damaged mitochondria on the pathway called mitophagy, a mechanism which is conserved across species.

AIM(S): One of the earliest signs of the disease are sleep disturbances, which appear before any motor symptoms. In this study, we used fruit fly, Drosophila melanogaster to investigate the effect of park mutation on clock neurons. The main oscillator is located in the sLNv neurons, which specifically express Pigment Dispersing Factor (PDF) and project their axons to the dorsal part of the brain. These terminals show circadian plasticity, being most branched in the morning and least at the evening, which provides different synaptic partners across the day. This mechanism regulates many processes, including the sleep and activity pattern.

METHOD(S): To examine how mitophagy disruption affects clock neurons, we used flies with a park mutation and with park silenced in sLNv and dopaminergic neurons. We analyzed activity and sleep profiles to check whether PD model flies have circadian disruption. We collected heads at two time points, at the beginning of the day and of the night and using anti-PDF immunostaining we visualized terminals to analyze their complexity using Sholl method.

RESULTS: The obtained results were very similar across all three experimental genotypes. They showed lower amplitude of activity during the morning and evening peak. Moreover, we observed disrupted rhythmicity in branching complexity.

CONCLUSIONS: Our results suggests that mitophagy disruption causes clock dysfunction, however the mechanism is more complex. It is possible that increased oxidative stress directly in clock neurons, or in neighboring cells desynchronizes sLNv physiology and in effect affects activity and sleep pattern.

FINANCIAL SUPPORT: NCN grant no. UMO-2022/46/E/NZ3/00095.

P1.45. HALLUCINATIONS AS A CONSEQUENCE OF PREDICTIVE CODING AND BAYESIAN INFERENCE DISRUPTIONS: A SYSTEMATIC REVIEW ACROSS PSYCHOSIS, PSYCHEDELICS, AND SENSORY DEPRIVATION WITH MACHINE LEARNING PERSPECTIVES

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INTRODUCTION: Hallucinations are perceptions without any external stimuli, which may arise from disrupted predictive coding, which is a neurocomputational framework deeply rooted in Bayesian inference. In this, the brain integrates prior beliefs with sensory input to minimize errors in prediction. When this mechanism breaks down due to very strong priors in psychosis, hypopriors in psychedelic states, and lack of input in sensory deprivation, hallucinations occur. Despite differences, these states share inferential dysfunction. ML can help us classify and predict the hallucinatory state by detecting patterns in EEG, fMRI, and other data, such as behavioural data.

AIM(S): This systematic review aims to identify and analyze studies exploring predictive coding and Bayesian inference disruptions in hallucinations across different states, compare how these mechanisms vary, and then evaluate the possible role of ML in classifying or predicting hallucinatory phenomena.

METHOD(S): A search was conducted on PubMed and Scopus (2020-2025) using PRISMA guidelines. Inclusion criteria covered empirical, computational, or ML studies focused on hallucinations that involved Bayesian and predictive mechanisms. A total of 46 studies met the inclusion criteria, which are included in this systematic review.

RESULTS: Studies show predictive coding disruptions as the main reason for hallucinations. In schizophrenia (impaired precision and a reduction in mismatch negativity), Psychedelics (hypopriors with unstable percepts), and Sensory deprivation trigger spontaneous top-down predictions. ML models using EEG and fMRI features like N100 suppression and effective connectivity may achieve high classification accuracy of hallucinatory states. However, inter-condition robustness remains limited.

CONCLUSIONS: This review sports a unified Bayesian framework underlying hallucinatory states. The integration of Bayesian inference with ML offers a promising path towards diagnostic biomarkers and precision interventions across altered states.

FINANCIAL SUPPORT: No financial support was received for this study.

P1.46. KETAMINE INDUCED REORGANIZATION OF BRAIN ACTIVITY: SPIKE ACTIVITY AND CHANGES IN GAMMA AND HIGH-FREQUENCY OSCILLATIONS

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INTRODUCTION: Fast oscillations recorded in local field potentials (LFP), such as gamma oscillations (30–100 Hz) and high-frequency oscillations (HFOs, 130-180 Hz), are thought to temporally coordinate spiking activity. Ketamine is a psychoactive compound currently under extensive investigation for its antidepressant potential. In rodents, the olfactory bulb (OB) exhibits large-amplitude HFOs following ketamine administration.

AIM(S): How ketamine-induced changes in gamma and HFOs relate to neuronal spike activity.

METHOD(S): LFP data from freely moving rats (N=7) from the OB were acquired using a SmartBox (NeuroNexus, A8 × 8-10 mm-200–200–177). Spike activity were detected with Kilosort4 package. Rats were recorded 20-minute baseline period and 30 minutes of post-injection recording (ketamine, 0.1 mg/kg).

RESULTS: After ketamine administration, a decrease in both gamma bands power and a noticeable increase in HFOs power were observed. Modulation analysis indicated that high-gamma activity was largely replaced by HFOs, implying a reorganization of oscillatory dy-

namics. CSD analysis, overlaid on the OB histology reveals a distinct spatial patterns for high gamma after ketamine. The peri-stimulus time histogram analysis identified two spike clusters: one showing synchronized activity with gamma oscillations during the baseline and another lacking of such synchronization. Interestingly, spike activity in both clusters coordinated with HFOs after ketamine treatment. Spike-field coherence analysis verified increased spike-phase locking within the post-ketamine administration, indicating improved synchronization between spiking activity and HFOs.

CONCLUSIONS: Ketamine-induced brain oscillations may alter cognitive function by balancing gamma and HFOs activity. The change from gamma synchronization to extensive HFOs may lead to less specificity in brain communication, resulting in altered information processing.

FINANCIAL SUPPORT: The study was funded by NCN (grant 2021/41/B/NZ4/03882).

P1.47. CALMODULIN CONTROLS SPATIAL AND TEMPORAL SPECIFICITY OF CALCIUM TRANSIENTS

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INTRODUCTION: Roughly half of dendritic spines, which comprise post-synaptic site of excitatory synapses, contain endoplasmic reticulum (spine apparatus, SA). Ryanodine receptors (RyR), which are activated by calcium (Ca), located in spine necks acts as a transducer of Ca signals from the spine to the dendrite. Out of the 3 types of RyR RyR2 are inhibited by calmodulin (Cam) and RyR3 are activated by Cam, which is one of the most abundant proteins in the brain. In control conditions in the brain both types of RyR are most likely constitutively bound to Cam. However, with higher oxidation levels Cam affinity for RyR is much lower. Dendrites of CA1 pyramidal neurons contain RyR2 and SA contain RyR3.

AIM(S): We wanted to confirm that SA acts as a transducer of Ca transients in control conditions and investigate the effect of higher oxidation levels accompanying old age on spatial and temporal specificity of Ca transients.

METHOD(S): We developed a multi-compartment stochastic reaction-diffusion model of signaling pathways underlying Ca regulation and Ca-induced Ca release in a 51 um long apical dendrite of a CA1 pyramidal neuron. For the condition of old age, which is accompanied by higher oxidation levels, we over-expressed RyRs, lowered activity of Ca extrusion pumps in the plasma membrane, doubled Ca buffering, and lowered cam affinity for RyR.

RESULTS: In control conditions only spines containing SA allowed for transduction of Ca transients from the spine to the dendrite. Spatial extent of those transients was symmetrical and reached roughly 5 um. In old age condition both types of spines allowed for transduction of Ca transients, which were also prolonged. Ca transients originating in spines without SA had spatial extent of 5 um. In old age condition spatial extent of Ca transients originating in spines with SA was doubled.

CONCLUSIONS: Calmodulin controls spatial and temporal specificity of calcium transients by inhibiting RyR2 receptors.

P1.48. CHARACTERIZATION OF THE CEREBRAL CORTEX IN THE NON-HUMAN PRIMATE BRAIN: A DEEP LEARNING MODEL INTERPRETABILITY APPROACH

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INTRODUCTION: Understanding how the cerebral cortex processes information involves laborious and knowledgeable characterization of its cytoarchitectonic properties. While it has been investigated for over a century, there is still no consensus regarding its structural and functional parcellation. Ongoing development of deep learning techniques provides promising support in the rapid processing of large amounts of high-resolution microscopic images, bringing a chance to alleviate these obstacles.

AIM(S): The study has two primary goals: to expand knowledge about the common marmoset (Callithrix jacchus) cerebral cortex features through quantitative characterization based on observer-independent segmentation, and to identify delineation criteria established by the U-Net deep learning model while assess-

ing its biological plausibility against expert neuroanatomical knowledge.

METHOD(S): To ensure a valid delineation of the cortex into layers and areas, we: (1) estimated neuronal density and size, (2) extracted one-dimensional cortical profiles, (3) trained a deep-learning model to segment and classify cortical profiles, (4) applied Gradient-weighted Class Activation Mapping (Grad-CAM) to investigate model's decisions, and (5) measured cortical depth along 3D profiles to quantify laminar thickness and spatial organization across the cerebral cortex.

RESULTS: The model applied to a dataset of Nissl-stained coronal sections of the marmoset brain was able to recognize layers and assist in manually performed delineation. Further evaluation revealed increased performance when neural density and size estimates contributed to the training process. Additionally, the model was capable of correctly assigning area labels to each profile based on learned features.

CONCLUSIONS: Beyond automating identification and quantification of individual layers and areas, our solution provides valuable insight into cytoarchitectonic properties by highlighting the most contributive parts of the cerebral cortex.

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P1.49. CAUSALITY IN NEURONAL CIRCUITRY, FROM GRANGER TO LLMS

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INTRODUCTION: Neuroscience circuitry at any scale has always involved time series analysis or even causality, where we try to discover the influence that a brain region or a group of neurons exerts over time. This is particularly relevant if we want to understand diseases that involve the transfer of information.

AIM(S): We present an end-to-end AI framework for directed graphs, incorporating explainable AI techniques, aimed at modeling brain connectivity in stroke patients. Additionally, we explore the integration of time series analysis using foundation models inspired by large language models to enhance temporal dynamics understanding.

METHOD(S): Those machine learning pipelines combine different types of causal estimators, such as Granger causality, or reservoir computing, combined with directed graph analysis to highlight even more differences between healthy subjects and subjects with trauma or diseases. Directed graphs are constructed from these connectivity measures and classified using a directed graph convolutional network. Explainable AI tools are employed to interpret the disrupted brain networks and identify relevant biomarkers. The inclusion of foundation model-based time series analysis enhances the temporal resolution and robustness of the connectivity features. Ultimately, a general framework is proposed to discover causality from MRI, EEG time series or other types of data.

RESULTS: The proposed framework achieved a classification level where the reservoir computing was relatively superior to the traditional Granger Causality. Time series foundation models have also been shown to be an alternative tool for time series prediction.

CONCLUSIONS: This approach demonstrates the potential of combining reservoir computing, directed graph analysis, foundation model-driven time series analysis, and explainable AI to improve patient stratification in brain diseases. Our technical innovations advance the understanding of effective brain connectivity and pave the way for more interpretable AI-driven clinical tools.

FINANCIAL SUPPORT: This work was supported in part by the Polish High-Performance Computing Infrastructure PLGrid (HPC Centers: ACK Cyfronet AGH) under Grant PLG/2024/017108; in part by the project of the Minister of Science and Higher Education ''Support for the Activity of Centers of Excellence established in Poland under Horizon 2020'' under Contract MEiN/2023/DIR/3796; in part by the European Union's Horizon 2020 Research and Innovation Program under Grant 857533; and in part by the International Research Agendas program of the Foundation for Polish Science, co-funded by the European Union under the European Regional Development Fund.

P1.50. OMENTIN-1 IS REGULATED BY KISSPEPTIN AND ESTRADIOL AND MODULATES GNRH SIGNALING VIA PKC, ERK1/2, AND CAMP PATHWAYS IN MOUSE GT1-7 NEURONS

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INTRODUCTION: The hypothalamus regulates hormone release critical for reproduction, growth, metabolism, and stress. Omentin-1 (OMNT1) is an adipokine that acts by insulin receptor (INSR) and involved in metabolic and reproductive regulation, but its role in central neuroendocrine control remains unknown.

AIM(S): This study aimed to investigate the expression and regulation of OMNT1 in hypothalamic neurons, and its role in neuroendocrine function and intracellular signaling pathways.

METHOD(S): The GnRH-secreting mouse hypothalamic cell line GT1-7 was used as an in vitro model. Firstly, OMNT1 localization with GnRH and the INSR was assessed by immunocytochemistry. Secondly, GT1-7 neurons were treated with kisspeptin (10, 100 and 1000 nM) or estradiol (1, 10 and 100 nM) for 24 h to measured OMNT1 protein expression by western blot. Next, effect of OMNT1 (10, 50 and 100 ng/mL) was studied on GnRH secretion (ELISA) for 15 and 30 min or GnRH expression (RT-qPCR, western blot) for 24 h. Moreover, OMNT1 effects on ERK1/2, PKC (western blot), and cAMP (ELISA) were determined. Using pharmacological inhibitor of ERK1/2 (PD098059 10 μM), PKC (Bisindolylmaleimide I,2 nM) or cAMP (SQ22536, 150 μM) GnRH secretion induced by OMNT was measured. Data were analyzed by Student's t-test or one-way ANOVA with Tukey's *post hoc* test ($p<0.05$; $n=6$).

RESULTS: OMNT1 expression was confirmed in GT1-7 neurons and colocalized with both GnRH and the INSR. Kisspeptin and estradiol modulated effect on OMNT1 protein levels. OMNT1 increased GnRH secretion after 30 min of incubation but reduced GnRH expression after 24 h. OMNT1 induced phosphorylation of ERK1/2 and PKC, while decreased intracellular cAMP levels. Inhibition of these signaling pathways confirmed their involvement in OMNT1-mediated GnRH secretion.

CONCLUSIONS: OMNT1 modulates GnRH neuron function via key signaling pathways, linking metabolic signals with reproductive control and providing new insight into adipokine action in the brain.

FINANCIAL SUPPORT: This research was supported by a grant from the Priority Research Area (Research Support Module) under the Strategic Programme Excellence Initiative at Jagiellonian University.

P1.51. THE EFFECTS OF SLEEP DEPRIVATION ON GUT CONDITION – DROSOPHILA MELANOGASTER MODEL

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INTRODUCTION: Maintaining a proper sleep schedule has been well proven to be crucial for our physical and mental wellbeing. Although there is a lot of research on how "pulling an all-nighter" can disrupt our brain functions, the importance of the connection between sleep hygiene and gut health remains mostly underexplored.

AIM(S): Given that disruptions in both sleep and gastrointestinal functions are observed in Parkinson's Disease (PD) this study aimed to investigate how one night's sleep deprivation affects the gut condition of Drosophila melanogaster park mutants. We investigated gene expressions levels, as well as the integrity of intestinal barrier following one night of sleep loss.

METHOD(S): All experiments were conducted on 5–7-day-old Drosophila melanogaster male flies. Gut samples were collected from the R4 region of the midgut. Each experimental group was subjected to sleep deprivation from 4:00 PM to 8:00 AM. Gut permeability was evaluated using the Smurf assay in park mutant flies. Gene expression levels of tim, npf, itp, and ninaD were analyzed in both head and gut tissues using quantitative PCR (qPCR).

RESULTS: We found that itp gene expression level was significantly higher in both heads and guts of the experimental group compared to the control. Moreover, after sleep deprivation expression of ninaD in the heads and tim in the guts were elevated. We also found smurf-positive flies to be present in only sleep-deprived group of park mutants, which indicates compromised intestinal barrier function in this particular group.

CONCLUSIONS: The elevation of genes involved in circadian regulation (tim) and water homeostasis (itp), suggests that sleep deprivation may disrupt homeostatic pathways critical for maintaining gut health. Those findings support the growing recognition of the gut-brain axis, and highlight how disturbances in sleep may exacerbate pathological processes relevant to PD development. Further research is needed in order to establish a molecular mechanism responsible for observed changes.

P2.01. A ROLE OF ASTROCYTIC IGFBP2 IN DENDRITIC SPINE STRUCTURAL PLASTICITY

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INTRODUCTION: Insulin-like growth factors 1 and 2 (IGF1 & 2) together with their receptor (IGF1R) have emerged as a critical autocrine system regulating dendritic spine structural plasticity. However, outside of the cell, IGF exists mostly in a complex with its binding protein 2 (IGFBP2). IGFBP2 which seems to be released mostly by astrocytes prevents IGF from degradation but also from binding to the IGF1R.

AIM(S): Here we aim to describe an extracellular system which involves Matrix Metalloproteinase- 9 (MMP-9) release from neurons, digestion of astrocytic IGFBP2 and release of IGF1 leading to IGF1R activation and synaptic plasticity.

METHOD(S): We have combined two-photon imaging and glutamate uncaging in to show structural long-term potentiation (sLTP)of dendritic spines in organotypic hippocampal cultures. We have used transgenic models of MMP-9 knockout as well as CRISPR/Cas9 knockdown of astrocytic IGFBP2 to elucidate role of those proteins in described system. Additionally, we have used immunofluorescent staining to localize IGFBP2 and validate gRNAs leading to IGFBP2 knockdown.

RESULTS: Using immunostaining we show that IGFBP2 is astrocytic protein and that it can be found in vicinity of synapses. We identify gRNAs leading to knockdown of IGFBP2. We show that blocking of IGFBP2 either with broad chemical inhibitor or with specific antibody reduces sLTP and that this effect can be rescued by application of IGF1. We show that MMP-9 cleaves IGFBP2 and is able to release IGF1. Moreover we show that MMP-9 knockout has decreases sLTP, which can be rescued by application of IGF1.

CONCLUSIONS: 1. IGFBP2 is a astrocytic protein which plays crucial role in sLTP. 2. IGFBP2 is important as a reservoir of IGF1 outside the cell 3. MMP-9 cleaves IGFBP2, leads to IGF1 release from the complex and activation of IGF1R.

FINANCIAL SUPPORT: National Science Centre, Poland grant: 2023/51/B/NZ4/02135.

P2.02. ASTROCYTE-DERIVED SYNAPTIC PROTEINS MEDIATE STRESS-INDUCED BEHAVIORAL CHANGES AND MODULATE ANTIDEPRESSANT RESPONSES IN MICE

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INTRODUCTION: Depression is one of the most prevalent mental health conditions worldwide, characterized by a diverse range of clinical symptoms, including impaired emotional regulation, social interactions and decision-making. While extensive evidence implicates disturbed synaptic homeostasis in the pathophysiology of depression, the role of astroglia in regulating plastic changes of neural networks in the adult brain remains underexplored. We previously found that chronic stress induces profound changes in transcriptomic profile of prefrontal cortex (PFC) astrocytes, which were mediated by glucocorticoid receptor (GR). Among stress-regulated genes were astrocyte-specific factors involved in synapse formation and elimination.

AIM(S): Here, we investigated how manipulation of genes controlling synapse number influences behavioral effects of chronic stress and how these genes contribute to antidepressant action.

METHOD(S): To achieve astrocyte-specific protein silencing in the mPFC, we bilaterally injected mice with shRNA-GFP vectors targeting each gene. Behavioral phenotyping was conducted using a system for tracking freely moving mice cohorts. Chronic corticosterone (CORT, 21 days in drinking water) was used to induce depressive-like behavior. We assessed individual and social behaviors in both sexes during three sessions: 3 weeks post-vector injection, post-stress, and 24h after a single subanesthetic ketamine dose. Immunostaining for GFP and astrocyte markers validated silencing efficacy and distribution.

RESULTS: Principal Component Analysis (PCA) of behavioral traits revealed that silencing of specific astrocytic proteins distinctly altered the behavior of animals following chronic CORT and ketamine exposure. We further identified the behavioral variables contributing most to the observed variance in PCA.

CONCLUSIONS: These findings underscore the pivotal role of astrocyte-derived synaptic proteins in GR-dependent stress mechanisms, advancing our understanding of how astroglia modulate neural circuits in depression.

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P2.03. FOCAL ADHESION KINASE MODULATION COUNTERACTS DENDRITIC SPINE REMODELING INDUCED BY CORTICOSTERONE IN CORTICAL AND HIPPOCAMPAL NEURONS

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INTRODUCTION: Dendritic spines are the primary sites of excitatory synaptic input, and their intact morphology is critical for the effective signal transmission. Prolonged elevation of corticosterone (CORT) is one of the factors that affects spine structure and, consequently, dysregulates synaptic function; however, the underlying mechanisms remain unclear.

AIM(S): Therefore, we investigated whether focal adhesion kinase (FAK)—known to influence spine remodeling and respond to CORT stimulation—contributes to CORT-induced structural changes of spines.

METHOD(S): Primary cortical and hippocampal cultures, rich in FAK and sensitive to chronic CORT, were exposed to 250 nM CORT for 72 h to induce and assess its effects on spine morphology. Additionally, a FAK inhibitor or activator was co-applied with CORT for 72 h to determine whether altering FAK activity could mimic or counteract CORT-driven remodeling. Finally, FAK mRNA and protein levels were measured after 48 h and 72 h of CORT to clarify FAK's involvement in the CORT-related signaling pathways that lead to spine remodeling.

RESULTS: 72 h of elevated CORT affected spines of cortical and hippocampal neurons differently, resulting in mild elongation of cortical spines and shortening of hippocampal spines. In contrast, the spine head width decreased in both types of neurons. FAK inhibition replicated these CORT-induced changes in cortical neurons but did not affect hippocampal spines. Conversely, FAK activation blocked all CORT effects on cortical spines and prevented the reduction in hippocampal spine head width, but not in length. After 72 h, but not 48 h of CORT, cortical FAK mRNA increased, while FAK protein remained unchanged. Hippocampal FAK mRNA and protein were stable at both time points.

CONCLUSIONS: Our results revealed a potential use of FAK activity modulation in preventing CORT-induced spine alterations. However, FAK is unlikely to mediate CORT signaling, which leads to spine remodeling.

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P2.04. REGULATED EXOCYTOSIS IN ASTROCYTES

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INTRODUCTION: The original concept of the tripartite synapse assumes that gliotransmitters are released on a way of exocytosis. Despite years of research, the involvement of astrocytic regulated exocytosis in synaptic processes remains a matter of debate. For instance, there is still no clear link between Ca^{2+} signaling in astrocytes and gliotransmission.

AIM(S): We aim to verify if regulated exocytosis exists in astrocytes and what are the mechanisms regulating this process.

METHOD(S): We used mix hippocampal culture of neurons and astrocytes. To monitor exocytosis of small vesicles, we have used genetically encoded exocytosis sensor consisting of VAMP2 in fusion with pH-dependent GFP (Superecliptic pH-luorin). We have used Total Internal Reflection Fluorescence (TIRF) Microscopy in combination with electrical stimulation of the culture, to elucidate neuronal-activity dependence of astrocytic exocytosis.

RESULTS: Our results demonstrate that the rate of exocytosis is significantly lower in mix cultures compared to pure astrocytic cultures, which have been used in most previous studies. Furthermore, using electrical stimulation, we show that neuronal activity increases the frequency of exocytosis and that this process can be blocked by TTX. Our findings indicate that exocytosis in astrocytes is calcium-dependent, with the primary source of Ca^{2+} being extracellular rather than from the endoplasmic reticulum. Additionally, we highlight the involvement of AMPA receptors in the regulation of exocytosis.

CONCLUSIONS: Our findings provide insight into certain mechanisms of exocytosis regulation in astrocytes that warrant further investigation.

FINANCIAL SUPPORT: National Science Centre, Poland grants: 2017/26/D/NZ3/01017 and 2023/51/B/NZ4/02135.

P2.05. GLUCOCORTICOID RECEPTOR SIGNALING ORCHESTRATES CIRCADIAN RHYTHMS IN BRAIN CELLS THROUGH CELL-INTRINSIC DYNAMICS

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INTRODUCTION: Physiological activity of neural networks is coupled to metabolic status of the tissue controlled by astrocytes. Multiple studies have shown that genes encoding proteins relevant for tissue metabolism show circadian oscillations. Glucocorticoids (GCs) are key circadian regulators, aligning transcriptional and metabolic processes across tissues. However, GC control of brain metabolism, in particular astrocytes, remains unclear.

AIM(S): To address this gap, we developed a live-cell imaging platform to study circadian rhythms in neural cells.

METHOD(S): Using fluorescent reporters driven by core clock gene promoters (Bmal1-mVenus, Cry1-mVenus), we monitored real-time oscillations.

RESULTS: Astrocytes showed robust circadian rhythms upon stimulation with the glucocorticoid receptor (GR) agonist dexamethasone (DEX, 100 nM), with a ~26-hour phase. A 2-hour DEX pulse induced oscillations lasting at least 3 cycles. In contrast, continuous 72-hour exposure dampened the 2nd and 3rd peaks, suggesting adaptive suppression of the feedback loop. This data suggest cell-autonomous mechanisms of circadian clock oscillations in the brain. We also studied the potential of several hormones and neurotransmitters for inducing the rhythmicity. Forskolin (FSK, 10 μM) induced oscillations under both constant and 2-hour exposure. However, melatonin (100 nM), serotonin (10 μM), adenosine (1 μM), acetylcholine (100 nM), and norepinephrine (50 nM) did not elicit clock gene oscillations in astrocytes.

CONCLUSIONS: GR activation induces distinct, cell-type-specific circadian oscillations in brain cells. The platform developed within this project will be used to explore GR-mediated regulation of cell-specific metabolites, advancing our understanding of stress-related disruption of brain metabolism.

FINANCIAL SUPPORT: This work was funded by the Industry PhD Grant- Tansu Göver (DWD/6/0306/2022) from Polish Ministry of Science and Higher Education, Norwegian Financial Mechanism 2014-2021 operated by the Polish National Science Center under the project contract 020/37/K/NZ3/02783 and The Horizon Europe Research and innovation funding programme under Grant Agreement 101079181 – SAME – NeuroID.

P2.06. TRANSCRIPTIONAL SIGNATURES OF HUMAN NEURAL CELLS TO GLUCOCORTICOID STIMULATION

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INTRODUCTION: Mental health disorders are leading contributors to the global disease burden, with chronic stress and stressful life events acting as major risk factors in disease etiology. The molecular mechanisms of the underlying pathology are not fully understood, limiting the development of innovative treatments. The stress response is mediated by glucocorticoids, which induce systemic physiological changes via the glucocorticoid receptor (GR), a ubiquitous transcription factor acting in a tissue- and cell-specific manner. Molecular pathways affected by altered GR signaling are well recognized in peripheral organs. However, the response to GR stimulation in the brain remains be elucidated.

AIM(S): Our research aims to uncover cell-type-specific pathways regulated by GR signaling in the context of human genetics and their implications in stress-related disorders.

METHOD(S): To this end, we exploited human induced pluripotent stem cells differentiated into surface-attached tridimensional mixed cultures of neural cells. Mixed cultures were treated with GR agonists (2.5 μM cortisol or 100 nM dexamethasone) and analysis of GR bona fide target genes was performed.

RESULTS: Immunocytochemical staining and qPCR analyses confirmed the presence of cortical neurons, astrocytes, and oligodendrocytes in both mature and progenitor states, along with GR expression in each cell type. The patterns of GR-activated transcription were characterized and compared with a large collection of GR-dependent transcriptional signatures. Further analysis revealed cell-type-specific components of the response.

CONCLUSIONS: This study offers deeper insights into the molecular underpinnings of stress-related psychiatric disorders and may support the identification of novel therapeutic targets.

FINANCIAL SUPPORT: This work was supported by NCN OPUS grant GRtraits nr 2022/45/B/NZ5/03188.

P2.07. MODULATING MICROGLIAL AUTOPHAGY WITH IBRUTINIB: INSIGHTS FROM IN VITRO PHARMACOLOGY

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INTRODUCTION: Bruton's tyrosine kinase (BTK) is key in B-cell signaling, autoimmunity, and inflammation, and may regulate autophagy. Ibrutinib, an FDA-approved BTK inhibitor, modulates immune cell function, including microglia. This project investigates how ibrutinib influences autophagy, oxidative stress, and neuroinflammation in glial cells, addressing a gap in neuroinflammatory disease research.

AIM(S): To investigate whether BTK inhibitor ibrutinib can show protective effects on mitochondria in glial cells in the LPS-induced model of neuroinflammation and explore the connection between mitochondria, BTK inhibition and autophagy.

METHOD(S): In-vitro cell treatment: Murine microglial cell line (C8-B4) and newborn Wistar rats aged 1 to 5 days were sacrificed through decapitation. Primary mixed glial cells were obtained from the cortices and hippocampi of postnatal (P1–P2) rat pups and used for the in vitro cell culture. Western blot Western blot experiments performed as per standard protocol and multiple autophagy and inflammatory markers were used to detect targeted parameter.

RESULTS: Our study shows that ibrutinib modulates LPS-induced microglial activation by lowering TLR4 and NF-κB cytokine levels as well as activate autophagy . This suggests the involvement of the TLR4/NF-κB pathway Ibrutinib's modulation of these pathways may help restore normal inflammatory responses. Despite extensive research, inconsistencies remain in understanding microglial activation and polarization, and limited data exist on the role of ibrutinib or BTK inhibitors in microglial oxidative stress.

CONCLUSIONS: Therapeutic targeting of BTK by its inhibitors in inflammatory CNS disorders or treatment of autoimmune diseases is an emerging strategy that holds huge potential and can support current treatments of several neurological disorders and bring new paradigms toward brain disease.

FINANCIAL SUPPORT: This work was supported by Charles University project Cooperatio – Pharmaceutical Sciences and Charles University Grant Agency (GA UK) 162224.

P2.08. THE OVEREXPRESSION OF PGC1A SUPPORTS NEURONAL MATURATION IN DORSAL FOREBRAIN ORGANOID MODEL

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INTRODUCTION: The PGC1α protein, encoded by the PPARGC1A gene, is a transcriptional coactivator regulating energy metabolism. Recently, several studies suggested the importance of the PGC1α in the development of neural system. Due to its role in promoting mitochondrial biogenesis, this protein may have therapeutic potential in neuronal disorders characterized by mitochondrial dysfunction.

AIM(S): The aim of this study was to investigate the effects of PGC1α overexpression on the neuronal differentiation of human dorsal forebrain organoids.

METHOD(S): Human induced pluripotent stem cells (iPSCs) were genetically modified using lentiviral vectors carrying either the CMV-GFP-puro control plasmid or the CMV-PPARGC1A-puro plasmid to achieve overexpression of PGC1α. After puromycin selection of the iPSCs, the expression of pluripotency markers was assessed by qRT-PCR and immunofluorescence staining. Subsequently, dorsal forebrain organoids were generated from the modified iPSC lines and collected on day 100 of development. Neuronal marker expression was analyzed by qRT-PCR and immunohistochemistry.

RESULTS: Analysis of PPARGC1A expression confirmed the successful generation of an iPSC line with increased levels of PGC-1α. The expression of pluripotency markers POU5F1 and NANOG remained unchanged, although SOX2 expression decreased in no significant manner. Dorsal forebrain organoids derived from engineered line revealed elevated expression of PPARGC1A as compared to controls. The expression of neural progenitor markers—NESTIN, CDH2, and SOX2 was not significantly altered. However, the expression of mature neuronal markers, FABP7, NEUROD1 and NEFL was increased in organoids with PPARGC1A overexpression.

CONCLUSIONS: The increased expression of PPARGC1A does not affect the expression of early neural

markers, but it promotes neuronal differentiation, suggesting that PGC-1α may support neuronal maturation in dorsal forebrain organoids.

FINANCIAL SUPPORT: This work was supported by the National Science Centre Grants No. 2022/45/N/NZ1/02754 and MMRI PAS.

P2.09. ACTIVATION OF TARCE-AMINE ASSOCIATED RECEPTOR (TAAR1): MOLECULAR CHANGES INDUCED BY TAAR1 AGONISTS

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INTRODUCTION: TAAR1 is a receptor for trace amines modulating dopaminergic, glutamatergic and serotoninergic neural transmission. In phase II clinical trials dual TAAR1 and 5HT1A receptor agonist, ulotaront, ameliorated both positive and negative symptoms of schizophrenia and did not cause extrapyramidal and metabolic side effects. Thus TAAR1 is an attractive therapeutic target for neuropsychiatric disorders. Molecular events induced by TAAR1 agonists are not fully elucidated. Recent studies suggest that activation of TAAR1 could induce Gαs, Gαq, Gαi and Gα12 or β-arrestin recruitment. Moreover, TAAR1 agonists could elevate ERK1/2 and CREB phosphorylation in PKA dependent manner. They could also activate Akt-GSK-3β pathway which may be involved in antipsychotic effect of current medicines.

AIM(S): Aim of this study is to reveal a signaling pathways induced upon TAAR1 activation by reference and novel compounds.

METHOD(S): We use Hit Hunter assay for studying cAMP signaling in cell line with overexpression of human TAAR1. Phosphorylation levels of ERK1/2, CREB, Akt and GSK-3β were measured using western blot analysis. β-PEA, ulotaront and novel TAAR1 agonist were selected for molecular studies. Cells were additionally treated with various inhibitors to determine cell signaling pathways involved in receptor activation.

RESULTS: Activation of TAAR1 by selected agonists results in increased intracellular cAMP and phosphorylation of ERK1/2 and CREB proteins which is blocked by PKA inhibitor. Additionally, elevation of Akt and GSK-3β proteins phosphorylation was observed.

CONCLUSIONS: Here we revealed that PKA regulates TAAR1 signaling induced by β-PEA, ulotaront and novel TAAR1 agonist. Moreover, we confirmed that TAAR1 agonists modulate activity of Akt and GSK-3β. Further studies are needed to elucidate the link between activation of these signaling events and antipsychotic effects of TAAR1 agonists.

FINANCIAL SUPPORT: Implementation doctorate program DWD/6/0431/2022; Celon Pharma S.A.

P2.10. MECHANOSENSITIVE PIEZO ION CHANNELS REGULATE OLIGODENDROCYTE MATURATION AND MYELINATION

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INTRODUCTION: Piezos are mechanosensitive transmembrane ion channels that convert mechanical stimuli into intracellular signals. In humans, Piezo1 and Piezo2 are primarily expressed in the cerebral cortex and white matter, respectively. Recent studies implicate Piezo channels in oligodendrocyte biology. Inhibition of Piezos using siRNAs or antagonists (Gd^{3+}, GsMTx4) shows neuroprotective effects and prevents chemically induced demyelination, while activation with Yoda-1 promotes demyelination and neuronal damage. Disruption of oligodendrocyte and OPC function in the CNS contributes to neurodegenerative diseases like AD, MS, PD, and HD. White matter alterations—particularly in the parahippocampal region and hippocampus—are linked to cognitive decline and memory deficits in AD highlighting the role of WM pathology and glial dysfunction in neurodegeneration.

AIM(S): to determine the expression profile of Piezo ion channels in OPCs throughout the stages of differentiation and myelination Examine do mechanical stimuli activate Piezo channels in OPCs and what downstream signaling cascades are engaged as a result How modulation of Piezo activity affects the morphological and functional development of myelinating cells.

METHOD(S): Primary OPCs are harvested from the cerebral cortices of postnatal day 4–7 C57BL/6 mouse pups. Cells are dissociated enzymatically and purified via magnetic-activated cell sorting (MACS) using anti-PDGFRα-coated beads.

RESULTS: Recent studies indicate that Piezo1 is expressed in OPCs and plays a complex role in myelination. Activation of Piezo1 with Yoda1 shown to induce demyelination and neuronal damage in ex vivo models, suggesting that overactivation of Piezo1 neg impacts myelination. Inhibition of Piezo1 using GsMTx4 attenuates demyelination and promotes remyelination in ex vivo and in vivo models, indicating a protective effect against myelin loss.

CONCLUSIONS: Our findings support a growing body of evidence that mechanosensitive Piezo channels are active participants in the regulation of myelination.

P2.11. DIVERGENT REGULATION OF MTOR AND RAPTOR IN MATURE HIPPOCAMPAL NEURONS DURING NUTRIENT STRESS AND SYNAPTIC ACTIVATION

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INTRODUCTION: The mechanistic target of rapamycin (mTOR) is a key regulator of neuronal metabolism, growth, and synaptic plasticity. While its role is well characterized in developing neurons, its nuclear behavior and regulation under metabolic stress in mature neurons remain poorly understood.

AIM(S): This study aimed to investigate the time-dependent response of mTOR signaling in mature hippocampal neurons during nutrient deprivation and to explore the nuclear localization patterns of mTOR complex component Raptor under both metabolic and synaptic stimulation.

METHOD(S): Primary hippocampal neurons from embryonic rats were cultured until maturity and subjected to nutrient deprivation in Neurobasal (NB) medium for 2 or 6 hours. Recovery was assessed by reintroducing full medium for 20 minutes. Immunocytochemistry was used to assess the levels and localization of mTOR, phospho-mTOR (P-mTOR), and Raptor. Kainic acid (KA) stimulation was used to mimic synaptic activity.

RESULTS: Short-term (2h) nutrient deprivation followed by refeeding resulted in a marked increase in mTOR and P-mTOR levels, indicating nutrient-sensitive activation. In contrast, prolonged (6h) deprivation led to elevated mTOR and P-mTOR levels even without refeeding, suggesting a shift in regulatory mechanisms. Raptor showed distinct nuclear puncta under control conditions and became more diffusely localized after KA stimulation. Raptor co-localized with nucleolin, indicating consistent nucleolar localization

CONCLUSIONS: mTOR signaling in mature neurons exhibits duration-dependent responses to nutrient stress. Raptor shows distinct nuclear localization dynamics, suggesting spatially and functionally separate roles from mTOR, particularly in response to synaptic activation.

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P2.12. DIFFERENTIAL BIOENERGETIC REMODELING BY TDP-43 DEPLETION REVEALS AMPK-MEDIATED MOTOR NEURON HYPERMETABOLISM

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INTRODUCTION: TDP-43 mislocalization and nuclear loss are early hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Paradoxically, patients with systemic metabolic disorders—such as type 2 diabetes and dyslipidemia—often exhibit slower ALS/FTD progression, suggesting that conventionally "risky" metabolic profiles may modulate TDP-43–driven neurodegeneration.

AIM(S): To delineate cell-specific bioenergetic effects of TDP-43 loss of function in motor neurons, neuronal cells, and microglia, and thereby uncover mechanisms underlying selective motor neuron vulnerability in ALS/FTD.

METHOD(S): TDP-43 was silenced by siRNA in NSC-34 motor neurons, N2A neuroblastoma cells, and BV2 microglia, with knockdown confirmed by Western blot. Twenty-four hours post-transfection, glycolytic flux

(extracellular acidification rate) and mitochondrial respiration (oxygen consumption rate) were measured using a Seahorse XF Analyzer. AMP-activated protein kinase (AMPK) activation was evaluated via Thr172 phosphorylation

RESULTS: In NSC-34 motor neurons, TDP-43 depletion induced concurrent increases in glycolysis and oxidative phosphorylation, defining a hypermetabolic phenotype. N2A cells exhibited reduced glycolytic and respiratory rates (hypometabolic), whereas BV2 microglia shifted toward predominantly glycolytic metabolism without altering respiration. Strikingly, only motor neurons showed elevated AMPK phosphorylation, implicating dysregulated energy sensing as a key driver of their selective vulnerability.

CONCLUSIONS: TDP-43 loss triggers cell-autonomous bioenergetic reprogramming: hypermetabolism with AMPK dysregulation in motor neurons versus divergent metabolic shifts in neuronal and glial lines. This mismatch may underlie selective motor neuron degeneration in ALS/FTD. Targeting AMPK pathways—and validating these findings in vivo—could yield novel neuroprotective strategies.

FINANCIAL SUPPORT: This study is supported by FNP and JPND.

P2.13. LIPID METABOLISM MODULATES MICROGLIAL PHAGOCYTOSIS OF AMYLOID-BETA

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INTRODUCTION: Alzheimer's disease (AD), the leading cause of dementia, is characterized by abnormal accumulation of amyloid-β (Aβ) in the brain. Aβ clearance is primarily the function of microglia, the brain-resident immune cells that are highly sensitive to environmental stimuli and respond to homeostatic changes via altering the release of inflammatory cytokines and phagocytosis. These functional adaptations in microglia are intricately linked to their metabolism, which provides a unique opportunity to harness microglial phagocytosis for selective Aβ clearance in AD without substantially harming healthy neurons.

AIM(S): To investigate how metabolic manipulation of microglia influences their capacity to phagocytose and degrade Aβ, with a focus on identifying mechanisms that enhance selective Aβ clearance without affecting healthy neuronal material.

METHOD(S): Aβ phagocytosis was tested in HMC3 human microglia following metabolic manipulation. Lipid starvation was induced by delipidation of the medium, and overall nutrient deprivation was achieved through serum starvation. Aβ uptake and degradation were quantified, and transcriptomic changes were assessed. Functional validation of candidate pathways was performed via knockdown of SREBF2.

RESULTS: Both lipid and serum starvation increased Aβ uptake in HMC3 microglia. However, efficient degradation of internalized Aβ was observed only under lipid starvation; serum starvation resulted in minimal degradation over 24 hours. Transcriptomic analyses revealed changes in pathways related to cholesterol biosynthesis, SREBF signaling, and steroid metabolism. Notably, knockdown of SREBF2 abolished the lipid starvation-induced enhancement of Aβ phagocytosis without impairing phagocytosis of healthy neurosynaptosomes.

CONCLUSIONS: These findings identify microglial SREBF signaling as a novel and selective target for enhancing Aβ clearance in AD. Targeting this pathway may allow for preferential removal of pathological Aβ while sparing healthy neurons. In vivo validation and clinical correlation using serum samples from AD patients are currently underway.

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

P2.14. DIET-DRIVEN NEUROINFLAMMATION AND BRAIN INSULIN RESISTANCE: UNVEILING THE WESTERN DIET'S ROLE IN ALZHEIMER'S PATHOLOGY

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INTRODUCTION: The Western diet (WD), originating in the USA, is characterized by ultra-processed foods rich in simple sugars and saturated fats. It is a major risk factor for metabolic disorders, insulin resistance, and inflammation accelerating aging. Long-term WD consumption is believed to impair brain function, trigger neuroinflammation, and increase the risk of Alzheimer's Disease (AD). AD, the leading cause of dementia, is defined by Aβ plaque accumulation and neurofibrillary tangles composed of hyperphosphorylated tau. There are two AD forms: late-onset sporadic AD (SAD) and early-onset familial AD (FAD). Recent studies highlight the role of unhealthy diet in SAD represented of 95% cases.

AIM(S): This study aimed to determine whether the WD, as a modifiable lifestyle factor, promotes AD by inducing neuroinflammation and brain insulin resistance, and to compare its impact on SAD and FAD models to identify early targets for prevention.

METHOD(S): Male C57BL/6J wild-type mice (SAD model) and APPswe mice (Tg2576, FAD model) were fed WD or a standard diet (CTR) from 3 months of age and analyzed at 4, 8, 12, and 16 months. Neuroinflammation (P2RY12, CD68, GFAP), brain insulin resistance (p-IRS-1 Ser616), and AD hallmarks (p-Tau Thr231, APP, Aβ) were studied in the entorhinal cortex.

RESULTS: Results indicate the entorhinal cortex of wild-type mice is sensitive to metabolic changes, showing brain insulin resistance, impaired macrophage phagocytosis, early microglial activation, disrupted p-Tau localization, and reduced APP. In APPswe mice, WD mainly enhanced astroglial activity. Tau-related lesions were present in both models, while wild-type mice showed intensified Aβ labeling. Senile plaques appeared in APPswe mice.

CONCLUSIONS: Our findings highlight the Western diet as a key modifiable risk factor for neuroinflammation, brain insulin resistance, and Alzheimer's pathology, revealing distinct mechanisms in SAD *vs.* FAD and opening new avenues for targeted prevention strategies.

FINANCIAL SUPPORT: Polish National Science Centre grants: SONATA 2014/15/D/NZ4/04361, OPUS 2022/47/B/NZ7/03005.

P2.15. EVALUATION OF NEUROPROTECTIVE POTENTIAL OF CLASSICAL AND NOVEL ANTI-INFLAMMATORY COMPOUNDS FOR PARKINSON'S DISEASE – IN VITRO STUDY IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS

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INTRODUCTION: MIP001 is novel anti-inflammatory and analgesic drug with favourable safety profile even at high doses.

AIM(S): Since neuroinflammation is a significant contributor to pathogenesis of various neurodegenerative diseases, in this study we screened for potential neuroprotective effect of MIP001 in cellular models of Parkinson's disease.

METHOD(S): We compared the effectiveness of MIP001 (1-300 microM) with clinically used anti-inflammatory drug, ibuprofen (Ibu, 10-300 microM) against cell damage induced by hydrogen peroxide (H2O2), 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenylpyridinium ion (MPP+) in undifferentiated (UN-) and retinoic acid (RA-) differentiated human neuroblastoma SH-SY5Y cells. The neuroprotective effects were measured by biochemical assays at the level of cell viability (MTT reduction test) and cytotoxicity (LDH release test).

RESULTS: We demonstrated that MIP001 and Ibu at concentrations up to 100 microM when given for 24 and 48 hours did not evoke any detrimental effects in UN- and RA-SH-SY5Y cell viability. MIP001 at concentrations of 10-100 microM significantly attenuated the cytotoxic effect of MPP+ in UN- and RA-SH-SY5Y cells, whereas it was only slightly protective at concentration of 30 microM against the 6-OHDA evoked damage in UN-SH-SY5Y, and against H2O2-evoked cell death in RA-SH-SY5Y cells. We did not notice any protection mediated by Ibu in all tested cell damage models in both studied SH-SY5Y cell phenotypes.

CONCLUSIONS: Our results evidenced a neuroprotective potential of MIP001 against various cell damaging factors, with its higher effectiveness in apoptotic (MPP+), than oxidative stress-based (H2O2 and 6-OHDA) neuronal cell damage models. Thus it is highly reasonable to further investigate MIP001 neuropro-

tective potency in other cellular and animal models of PD.

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).

P2.16. EXPLORING THE ROLE OF THE ANTERIOR CINGULATE CORTEX IN ADHD- AND SCHIZOPHRENIA-RELATED DYSFUNCTIONS USING BEHAVIOURAL ANALYSIS, CHEMOGENETICS, MACHINE-LEARNING, OPTO- AND ELECTROPHYSIOLOGY

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INTRODUCTION: Circuit Neuroscience methods like chemogenetics, miniscope imaging and electrophysiology in mice undergoing behavioural testing allow the identification of cellular and molecular targets to modify cognitive or affective deficits of psychiatric disorders. We have conducted a series of studies using these techniques to identify potential targets to improve cognitive deficits seen in ADHD and schizophrenia.

AIM(S): Our aim was to identify cortical brain regions, cell types within those regions and signalling cascades within those cell-types that improve impulse control and attention.

METHOD(S): We used chemogenetic modulation of certain inhibitory and excitatory neurons, miniscope imaging and electrophysiological recordings in the 5-choice-serial-reaction-time task (5-CSRTT) which assess impulsivity and sustained attention, in mice. Machine-learning-based decoding and encoding analysis were conducted on opto- and electrophysiological data. Custom-made pyControl-based operant boxes and motorized commutators were developed to allow physiological recordings in operant testing.

RESULTS: Applying those techniques we found that: (1) Among prefrontal regions, the anterior cingulate (ACC) has the most robust influence on 5-CSRTT behaviour. (2) Activation of the Gq-protein cascade in inhibitory parvalbumin (PV) neurons of the ACC improves both impulse control and attention, and decreases gamma-oscillations in ACC – as does the ADHD medication atomoxetine. (3) Activation of the Gi-protein cascade in layer 5 pyramidal neurons (PNs) of the ACC decreases impulsivity, and this can be mimicked by activating mGlu2-receptores expressed in them. (4) ACC-PCs simultaneously encode the spatial motor plan (action) just before the choice and cognitive state during and after the choice during 5-CSRTT behaviour, and this encoding is modulated by reward value. (5) Chronic ketamine exposure increases beta/gamma-band input into ACC.

CONCLUSIONS: ACC controls impulsivity and attention, and could be targetted in ADHD or schizoprenia.

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P2.17. INTERDEPENDENCY BETWEEN OXYTOCIN AND DOPAMINE IN TRUST-BASED LEARNING IN MICE

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INTRODUCTION: Oxytocin (OT) is a neuropeptide implicated in complex social behaviors including trust and attachment, yet the neural mechanisms underlying its effects remain unclear. OT is thought to modulate behavior by enhancing the salience of social cues and attenuating prediction error (PE) processing, the discrepancy between expected and actual outcomes that drives learning.

AIM(S): Since both salience coding and PE processing involve dopamine (DA) neurons as well, the current study investigated the putative interdependence between OT and DA in social safety learning using the social transmission of food preference (STFP) paradigm. STFP is based on the observation that mice display neophobia toward novel food, but develop a preference for it after a conspecific demonstrator signals its safety.

METHOD(S): OT receptor activity was stimulated via OT administration and DA depleted using tetrabenazine (TBZ) in male mice, creating 3 experimental groups (OT, TBZ, OT+TBZ).

RESULTS: We interpreted STFP acquisition as a functional parallel to human trust-based learning and found that OT enhanced learning in a trust acquisition condition, but only when DA signaling was intact. In a trust violation condition, where the demonstrated food was paired with lithium chloride (LiCl)-induced nausea after the social interaction to induce a PE, both OT and DA depletion blocked learning, resulting in retained preference for the demonstrated food, but not when OT was administered under DA depletion.

CONCLUSIONS: These findings reveal a functional interaction between the OT and DA systems to modulate social safety learning, which may have important implications for OT's potential in treating disorders involving DA dysfunction.

FINANCIAL SUPPORT: FWO Flanders.

P2.18. THE SWITCHBOARD TEST: A FLEXIBLE, LOW-COST PLATFORM FOR STUDYING SPATIAL MEMORY IN RATS DURING SEQUENTIAL INSTRUMENTAL TASKS

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INTRODUCTION: Appetitive instrumental conditioning is widely used to shape animals' associations between actions and outcomes under controlled conditions. When implemented in automated environments with more complex tasks, it enables the collection of high-resolution data across hundreds of trials, supporting fine-grained behavioral analysis.

AIM(S): To develop and validate a flexible operant conditioning setup for assessing spatial memory in rats.

METHOD(S): We present an automated, low-cost environment for studying spatial memory in rats. The setup supports training and testing of location- and sequence-specific responses, with adjustable parameters such as layout, sequence length, cues, timing, and reward size. The 64 × 64 cm open field features nine floor buttons (3 × 3 grid) and a single reward area. An Arduino and PC manage switches, the dispenser, feedback sound playback, and a 64-LED reward cue. The system is compatible with electrophysiology and DBS, offering a flexible tool for behavioral research.

RESULTS: Over several weeks, rats (n=16) were gradually trained to memorize a target button-press sequence, which outlined a fixed spatial route within the arena. Each trial was self-initiated and, if successfully completed, ended with a distinct sound and a light cue, indicating reward delivery and signaling successful trial completion. Errors resulted in a distinct feedback sound and immediate termination of the trial. Upon activation, each switch in the target sequence triggered a unique sound from a set of complex natural sounds, such as those of crickets, small frogs, and dolphins, intended for further transfer of spatial memory to the auditory domain. In expert rats, removing all feedback sounds had a minimal effect.

CONCLUSIONS: Based on experimental data, we proposed a memory score calculation to generate learning curves, offering a sensitive metric of spatial memory performance. This study presents a novel approach to evaluating spatial memory within a flexible, dynamic operant conditioning framework.

FINANCIAL SUPPORT: This work was supported 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, Polonez Bis 3, project No. 2022/47/P/NZ4/03358; and by the Gdańsk University of Technology, Platinum IDUB grant: 1/1/2024/IDUB/I.1B/Pt.

P2.19. MODES OF SOCIAL COOPERATION IN RATS PERFORMING A SEQUENTIAL SPATIAL MEMORY TASK

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INTRODUCTION: Animals often coexist in shared environments where individuals have a similar ability to freely alter the state of the surrounding world. In such contexts, social strategies directly affect success in acquiring resources, as a lack of cooperation may impede each other's actions.

AIM(S): Our Study aimed to examine modes of social cooperation in rats within an instrumental conditioning framework.

METHOD(S): To test social strategies in rat pairs, we developed an automated setup for a sequential spatial memory task. The open field (64 × 64 cm) features nine floor buttons (3 × 3 grid) and a single reward area. The system uses an Arduino and PC to manage switches, reward delivery, sounds, and LED cues. Rats (n=16) were trained to memorize a three-button sequence and became experts. They were tested in 8 cagemate pairs (9 days), 112 unique intercage pairs (3 days each), 28 pairs with naïve cagemates, and 28 with naïve non-cagemates. Behavioral system data were combined with position tracking from video recordings processed using DeepLabCut, allowing us to determine each rat's behavior.

RESULTS: Our data suggest that, despite individual task proficiency, cooperation in a social context is a distinct skill that rats must develop and refine to succeed in an environment with a single reward-dispensing area. We observed different modes of operation, including cooperation, temporal segregation, and conflicts, which were resolved in various ways. We evaluated the effects of conflict on dynamic changes in social strategies. Additionally, we examined whether rats, over time, generalized this social skill to perform the task effectively with different partners, and whether extended social experience influenced their individual performance in the same task.

CONCLUSIONS: 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: This work was supported 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, Polonez Bis 3, project No. 2022/47/P/NZ4/03358; and by the Gdańsk University of Technology, Platinum IDUB grant: 1/1/2024/IDUB/I.1B/Pt.

P2.20. LOOKING FOR DISTINCT POPULATIONS OF CELLS CODING SOCIAL STIMULI IN CENTRAL AMYGDALA

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INTRODUCTION: The central amygdala (CeA) is a key structure in the brain's motivational system, playing a crucial role in processing social behaviors. However, the specific contributions of different neuronal populations to social stimuli processing in CeA remain poorly understood. A deeper understanding of how distinct cell types within the CeA respond to social interactions, as well as their connectivity with other brain regions, is essential for uncovering the neural circuits underlying social behavior. Such insights could provide valuable information for understanding social deficits associated with neuropsychiatric disorders and identifying potential therapeutic targets.

AIM(S): In this study, we aimed to characterize how distinct CeA neuronal populations contribute to processing social stimuli.

METHOD(S): We focused on two well-characterized inhibitory neuron types: somatostatin-positive (SOM)

and corticotropin-releasing factor-positive (CRF) neurons. To investigate their functional connectivity, we mapped both the input and output projections of these populations using transgenic SOM-Cre and CRF-Cre mouse models. Stereotactic surgeries were performed to introduce AAV DIO-mCherry for tracing output projections and rabies viruses for input mapping. Following recovery, mice were exposed to social stimuli, and neuronal activation was assessed by mapping cFOS-positive cells.

RESULTS: Our findings provide a detailed characterization of the input and output projections of SOM and CRF neurons in the CeA and their engagement during social stimuli processing. By linking neuronal activation patterns with anatomical connectivity, we identified key circuits involved in social behavior.

CONCLUSIONS: This study advances our understanding of the CeA's role in social information processing and lays the groundwork for future research into social deficits observed in conditions such as autism spectrum disorder and social anxiety.

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P2.21. MANIPULATION OF PREFRONTAL SOMATOSTATIN INTERNEURON ACTIVITY AFFECTS EMOTIONAL CONTAGION

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INTRODUCTION: The role of specific neuronal populations in the prefrontal cortex in emotion recognition is well characterized (Jabarin et al 2025). Specifically, somatostatin interneurons were (Choi et al. 2024, Dautan et al. 2024, Scheggia et al. 2020) indicated as playing a crucial role in this process. Their exact role in emotional contagion remains unknown.

AIM(S): To study the role of prefrontal somatostatin interneurons in emotional contagion occuring during remote transfer of fear paradigm.

METHOD(S): To do that mice were housed in pairs for three weeks, one labelled an Observer, and the other a Demonstrator. In the test session, the Demonstrator was subjected to aversive stimuli (10 foot shocks 0.6mA 1s long), outside of the home cage, while the Observer remained there undisturbed. Upon the return of the Demonstrator to the home cage, we recorded the interactions of the two animals. The behaviour was assessed with DeepLabCut (for pose estimation) and that set was then fed to Simba for detailed classification. Ninety minutes after the onset of the interaction animals were sacrificed for immunochemistry. To check the activity of somatostatin cells we either used mice expressing fluorescence marker (dTomato) in somatostatin cells whose brain slices were subjected to immunochemistry against c-Fos or checked that expression in neurons infected with pAAV-hSyn-DIO-HA-hM4D(Gi)-IRES-mCitrine (AAV8) or pAAV-hSyn-DIO-HA-h3D(Gq)-IRES-mCitrine (AAV8) in SOM-Cre mice.

RESULTS: We observed an increased expression of c-Fos in somatostatin cells in both pre and infralimbic prefrontal cortex of Observers subjected to a stressed cagemate. The chemogenetic excitation (c21) of somatostatin neurons resulted with a decrease of investigation of the anogenital region of the Demonstrator by the Observer, while inhibition of these neurons had no significant effect.

CONCLUSIONS: This proves that somatostatin interneurons are essential for regulation of emotional contagion in the safe environment of the home cage

FINANCIAL SUPPORT: NCN 2019/35/N/NZ4/01948 and 2022/47/B/NZ4/01192.

P2.22. THE DYNORPHIN– K OPIOID RECEPTOR SYSTEM SHAPES SOCIAL RECOGNITION MEMORY

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INTRODUCTION: Social memory—the ability to recognize and remember conspecifics—is essential for adaptive social behaviors. Previous studies using genetic and pharmacological approaches have demonstrated that both the inactivation of the prodynorphin gene ($Pdyn^{-/-}$) and the blockade of κ-opioid receptors

(KOR) enhance partner recognition in mice, while KOR activation impairs social memory. These findings suggest that reduced KOR signaling and the resulting neuronal disinhibition facilitate social memory processing. However, the specific neuronal populations expressing KOR that are involved in this process remain unclear.

AIM(S): The aim of this study was to determine whether selective deletion of KORs from oxytocinergic or serotonergic neurons affects social memory performance, and whether this is accompanied by changes in monoamine levels in the prefrontal cortex and striatum—key areas for reward and social behavior.

METHOD(S): We assessed social memory in genetically modified mice with selective deactivation of KOR on oxytocin (Oprk1OxtCre) or serotonin (Orpk1Tph-CreERT2) expressing neurons. Mice were exposed once to an unfamiliar, same-sex juvenile animal, and social memory was assessed by decreased interaction time with the same partner during a second encounter. Using high-performance liquid chromatography (HPLC), we also evaluated changes in monoamine concentrations within the prefrontal cortex and striatum.

RESULTS: Preliminary findings indicate that female Oprk1OxtCre mice exhibit prolonged social memory retention, resembling the phenotype observed in Pdyn$^{-/-}$ mice. In contrast, Oprk1Tph2CreERT2 mice displayed normal sociability and intact social memory.

CONCLUSIONS: Taken together, our results indicate a sex-dependent link between oxytocin and dynorphin signaling in social memory regulation, and suggest that KOR signaling on oxytocinergic neurons, rather than serotonergic neurons, is crucial in this process.

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P2.23. TCF7L2 DEFICIENCY IN THE THALAMUS LEADS TO ALTERATIONS IN THE SOCIAL BEHAVIOUR PROFILE

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INTRODUCTION: TCF7L2 is a transcriptional effector of the Wnt/β-catenin signaling pathway, which controls developmental and homeostatic processes and is a risk gene for autism spectrum disorder (ASD). TCF7L2 is expressed in the thalamus, where it regulates the establishment of thalamocortical connections and electrophysiological maturation of neurons. The role of TCF7L2 in regulating behavioural profiles has not been fully investigated.

AIM(S): We hypothesized that postnatal thalamus-specific deficiency of TCF7L2 impairs thalamocortical circuits and leads to autism-like behaviours.

METHOD(S): We investigated these hypotheses using mice with Tcf7l2 knockout mediated by Cre recombinase, whose expression was induced postnatally in thalamic neurons. We analyzed the behavioural profile of the conditional Tcf7l2 knockout (Tcf7l2 cKO) mice to assess social performance through Eco-HAB, Three-chamber, and Investigative interaction tests.

RESULTS: Tcf7l2 cKO presented a decrease in social interest, not interacting with other mice during the chamber exploration, spending less time near the social scent, and a decreased number of social contacts, demonstrating that the deletion of Tcf7l2 affects conspecific social cue recognition. After six weeks of ketogenic diet intervention, the majority of social parameters were rescued in cKO mice.

CONCLUSIONS: These results corroborate a hypothesis that thalamic dysfunctions originating from perinatal development can be a primary cause of social deficits. The response to the ketogenic diet suggests that impaired energy metabolism in thalamocortical circuits plays a role in the pathogenesis of ASD.

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P2.24. SAFEGUARDING OBJECT-DIRECTED BEHAVIOR: THE ROLE OF ENTORHINAL-AMYGDALA CIRCUITS

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INTRODUCTION: Object-directed behavior is essential for survival in natural environments. Although recent research has concentrated on how objects are encoded and egocentric spatial relationships are represented within the lateral entorhinal cortex (LEC), the downstream circuitry and mechanisms governing these behaviors remain largely unknown. Due to its connectivity a potential target could be the amygdala, known for driving defensive and appetitive behaviors; however, understanding how it integrates spatial object information remains challenging.

AIM(S): To address these gaps, we aimed to conduct a comprehensive LEC-amygdala network investigation for its role in object valence encoding and their distances, as well as experience-dependent avoidance behavior using a mouse object-conditioning task.

METHOD(S): We used CTB tracing and object conditioning task with c-fos mapping for a preliminary circuit screen. Next, a projection and/or cell-type-specific Ca2+ imaging and optogenetic manipulation during our behavioral task was used, followed by machine learning modeling of our circuit in driving the relevant behaviors.

RESULTS: We show the complexities of LEC-to-amygdala (BLA and CEl) projections, which are finely tuned to regulate spatial interactions with objects in the environment. These circuits incorporate 2 experience-dependent valence channels, which modulate an ethologically relevant safety mechanism that is crucial for navigating threats amid uncertainty. The balance between these signals exert precise control over object encounters and proximity.

CONCLUSIONS: Together, we propose a comprehensive neuronal circuit framework underlying ethologically relevant behavior and offer a roadmap for future exploration into the intricate interplay between action sequences and object encoding. This not only expands current understandings of LEC-amygdala circuitry function but also sheds light on the transformative process through which object information within the brain network translates into meaningful, directed behavior.

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P2.25. SWEET CONSEQUENCES: UNRAVELING THE IMPACT OF DIETARY SUGAR ON WEIGHT GAIN AND COGNITIVE FUNCTION IN MICE

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INTRODUCTION: Obesity is a global pandemic associated with an increased risk of life-limiting conditions such as metabolic syndrome. Two primary factors are widely considered to contribute to the development of obesity: unrestricted access to highly palatable foods and a sedentary lifestyle. Numerous studies also indicate a link between obesity and cognitive impairment, although the underlying mechanisms remain unclear.

AIM(S): Designing a behavioral test to detect subtle cognitive changes in mice subjected to different diets. The proposed test is based on a complex task and avoids the use of aversive or appetitive stimuli, in order to eliminate confounding stress or nutritional influences.

METHOD(S): Mice were fed one of four diets for eight weeks: standard (STAND), high sugar (HSD), high fat (HFD), or ketogenic (KD), characterized by progressively altered metabolized energy from carbohydrates or fats. Moreover, dietery sugar content followed a U-shaped pattern, the highest in HSD and HFD. Afterward, mice underwent a Thirst-Based Cognitive Test in the IntelliCage system, requiring corner visits in a set order – clockwise or counterclockwise – to gain access to plain water. Performance was measured as the ratio of correct to total number of visits.

RESULTS: At baseline, there were no significant differences in body weight among the groups. After eight weeks, the HSD and HFD groups displayed clear signs of obesity compared to the STAND group. Per-

formance in the thirst-based cognitive test varied by diet; mice in the HSD and HFD groups demonstrated impaired learning of the complex spatial task compared to the control group.

CONCLUSIONS: A short-term dietary intervention of eight weeks was sufficient to impair performance on a cognitively demanding task in mice fed HSD and HFD diets. These findings suggest that elevated dietary sugar intake correlates with deficits in cognitive function.

FINANCIAL SUPPORT: This work was supported by the ANIMOD project within the Team Tech Core Facility Plus program of the Foundation for Polish Science, co-financed by the European Union under the European Regional Development Fund and by National Science Centre (OPUS) grant 2019/35/B/NZ4/02831 (to W.K.).

P2.26. EFFECT OF IRON ADMINISTERED IN THE NEONATAL PERIOD ON THE BEHAVIORAL AND BIOCHEMICAL PARAMETERS IN ADULT RATS

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INTRODUCTION: Iron, a very important chemical element in the body of mammals, is the most abundant metal in the brain. It participates in many chemical reactions taking place in the central nervous system acting as a cofactor in key enzymatic reactions involved in neurotransmitter synthesis and degradation, dendritic arborization, and myelination Some available data indicated that concentration of iron in the brain progressively increases during the aging process in subjects, and it is selectively accumulates in the brains of patients suffering from neurodegenerative disorders. Iron-induced oxidative stress has been implicated in the pathogenesis of Alzheimer's, Parkinson's, and Huntington's disease, and others.

AIM(S): The aim of our study was to assess the influence of iron administered orally (30 mg/kg) to rats in the neonatal period (p12-p14) on the behavioral and biochemical parameters in adult rat.

METHOD(S): The tests were performance in adult rats in the behavioral tests; open field, social interaction tests, and recognition memory, and also in biochemical test, the BDNF mRNA expression in the cortex and hippocampus.

RESULTS: Iron administered to rats in the neonatal period induced long-term deficits in behavioral tests in adult rats. It reduced the exploratory activity in the open field test. In the social interaction test, it induced deficits in the parameters studied, and decreased memory retention. Moreover, it decreased the expression of BDNF mRNA only in the hippocampus.

CONCLUSIONS: The above data suggest the decreased BDNF mRNA expression by iron given in the neonatal period may play a role in iron-induced memory impairment in adult rats.

FINANCIAL SUPPORT: This study was financially supported by statutory funds of the Maj Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland.

P2.27. IMMOBILITY RELATED THETA RHYTHM IN THE POSTERIOR HYPOTHALAMIC AREA IN FREELY MOVING RATS

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INTRODUCTION: Theta rhythm is one of the most prominent examples of rhythmic oscillatory activity in the mammalian brain. It is generated mainly in structures of the limbic cortex, including the hippocampal formation (HPC). Theta rhythm is observed in humans during many physiological processes, such as spatial navigation, paradoxical sleep, and language processes. Considering the correlation with the animal's behavior, rodents' hippocampal theta rhythm was divided into two types: mobility-related and immobility-related rhythms. Studies from the 1970s showed, that theta rhythm may be also recorded in the posterior hypothalamic nuclei and supramammillary nucleus, together considered as the posterior hypothalamic area (PHa). Further studies have shown the presence of theta rhythm in local recordings from PHa during the performance of voluntary movements by rats.

AIM(S): The aim of present study was to verify whether the PHa is capable of generating, immobility-related theta rhythm in addition to mobility-related subtype described earlier.

METHOD(S): The experiments were conducted on 8 adult, male rats. Each animal was implanted with a 16-channel recording electrode in PHa, during stereotactic surgery. After 7 days of recovery, each animal was subjected to behavioral testing in the open field test. The animals were tested three times a week for the next 3 weeks, in the open field arena for 10min. During the test, radio-transmitted EEG recordings from PHa were collected in conjunction with video recording of each freely moving animal.

RESULTS: Obtained data indicated that theta rhythm can be recorded from PHa in freely moving rats not only during voluntary movements but also during immobility. Furthermore, there were significant differences in basic parameters between both kinds of that rhythmic oscillatory activity.

CONCLUSIONS: Considering the fact that HPC theta rhythm plays a significant role in multiple physiological conditions, further studies exploring the role of local theta activity in PHa seem relevant.

FINANCIAL SUPPORT: The research was funded by a grant from the National Science Center (PL): 2017/25/B/NZ4/01476.

P2.28. NEUROPROTECTIVE ROLE OF VOLUNTARY PHYSICAL ACTIVITY IN MICE C57BL/6 EXPOSED TO METHAMPHETAMINE

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INTRODUCTION: Methamphetamine (METH) is a potent psychoactive agent that affects the central nervous system, mainly by increasing neurotransmitter levels such as dopamine, norepinephrine and serotonin. Long-term use results in cognitive deficits and memory impairment.

AIM(S): The study aimed to test the hypothesis that voluntary exercise protects against METH-induced neurotoxicity and neurogenesis disruption in adult female and male mice.

METHOD(S): 10-week-old C57BL/6 mice, both male and female, were used in the experiment. Within each sex, mice were divided into two groups – METH and Saline (Veh). Mice received an injection three times a day, for five days, in 4-hour intervals. METH was administered according to an ascending dosage schedule (0.2 – 2.4 mg/kg), using increments of 0.2 mg/kg with each injection for 4 days. On the last day, mice were given METH in three doses of 4.0 mg/kg. Mice from the control group were injected with saline. After the injection procedure, mice were subjected to voluntary wheel running for 14 consecutive days, followed by behavioral tests measuring locomotor activity (Open Field Test), cognitive ability (New Object Recognition Test), and spatial learning (Morris Water Maze). Mouse brain slides were prepared from the hippocampal area and then analyzed by immunofluorescence.

RESULTS: METH induced impaired neurogenesis, and led to distruption of cognitive functions in mice, with a noticeable sex-related difference in the response to METH toxicity. Physically active mice showed less neuronal damage and better performance in cognitive tests than the sedentary ones. In running mice, increased neurogenesis was observed in the hippocampus, which was correlated with an improvement in memory and cognitive function.

CONCLUSIONS: METH primarily increases physical activity, nevertheless, in the long term leads to neuronal damage and cognitive deficits in mice. Our results indicate that humans addicted to METH may benefit from appropriate physical activity protocols.

FINANCIAL SUPPORT: Research funded by the National Science Center, Poland, grant no. 2019/33/B/NZ4/02721.

P2.29. THE ROLE OF LIPID METABOLISM AND CIRCULATING MIRNAS IN THE INTERGENERATIONAL TRANSMISSION OF THE EFFECTS OF PARENTAL ADVERSE CHILDHOOD EXPERIENCES

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INTRODUCTION: Childhood trauma is an important risk factor for psychiatric and physical ailments during adulthood. Emerging evidence suggests that some of its behavioral and metabolic symptoms are transmissible across generations. Intergenerational transmission of the effects of trauma is postulated to involve changes in germline non-coding RNAs. However, it is unclear how childhood trauma affects ncRNAs in the gametes. Circulating ncRNAs, such as miRNAs, majorly carried by lipid-associated factors in the body fluids, appear as important candidates for carrying the trauma effects to the gametes for intergenerational transmission.

AIM(S): Synergizing investigation in a mouse model of ACE induced via unpredictable maternal separation and unpredictable maternal stress (MSUS) and cross-injections, we hypothesize that lipid-associated miRNAs communicate the effects of ACE to the germline for intergenerational transmission.

METHOD(S): Intergenerational behavioral and metabolic phenotyping was performed, supplemented with small RNA sequencing followed by qPCR. Cross-injections of lipid-associated carriers into the tail vein of mice performed.

RESULTS: Offspring of both MSUS- and HFD-exposed male mice showed impaired glucose tolerance, depressive-like behavior and anxiety. Cross-injections from MSUS into CTRL mice prolonged the offspring latency to enter open arms in Elevated Plus Maze test. Cross-injections from MSUS into CTRL mice recapitulated the offspring metabolic phenotype associated with MSUS in Glucose tolerance test. Cross-injections from VE mice into MSUS mice partially mitigated the metabolic MSUS phenotype.

CONCLUSIONS: Injections of MSUS-material is sufficient and necessary to induce the intergenerational metabolic phenotype associated with MSUS while lipid-modifying interventions can potentially alter the intergenerational metabolic MSUS phenotype. This research provides proof-of-concept for a role of lipids and circulating miRNAs in communicating the effects of ACE to the germline for intergenerational sequelae.

FINANCIAL SUPPORT: LIPITRATE: National Science Centre (NCN) Poland (SONATA; DEC-2020/39/D/NZ3/01887).

P2.30. THE ROLE OF FPR2 IN THE AGE-RELATED CHANGES IN THYMUS-BRAIN AXIS

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INTRODUCTION: Formyl peptide receptor 2 (FPR2) is a G protein-coupled receptor involved in resolving inflammation and regulating immune and nervous system processes. Its dysfunction is associated with aging-related decline in both systems. One hallmark of immunosenescence is thymic involution—an age-related reduction in thymic size and function, which impairs T cell production. The thymus, essential for the clonal selection and maturation of T lymphocytes, gradually atrophies with age, yet continues to influence immune homeostasis by supporting regulatory T cell (Treg) generation in adulthood.

AIM(S): This study aimed to examine age-related changes in the thymus and brain in FPR2 knockout (FPR2KO) mice compared with wild-type (WT) controls.

METHOD(S): Comparative analyses were conducted in 15- and 18-month-old mice. Relative thymus, brain, and spleen masses were measured. Additionally, FPR2 protein levels were assessed by ELISA in the hippocampus and frontal cortex of WT mice from youth to aging.

RESULTS: Brain mass was significantly reduced in older mice, regardless of genotype. WT mice showed a notable decline in thymus mass with age, confirming thymic involution. In contrast, FPR2KO mice maintained higher thymus mass at both time points, suggesting attenuated involution. FPR2 levels in WT brains varied with age, indicating potential involvement in neural aging.

CONCLUSIONS: These findings suggest that FPR2 may play a key role in regulating thymic aging and maintaining brain homeostasis, linking immune and neural aging through pro-resolving pathways.

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P2.31. NEUROPROTECTIVE POTENTIAL OF BIFIDOBACTERIUM ANIMALIS CCDM 366 AND ITS BEVS: IMPACT ON THE BLOOD-BRAIN BARRIER FUNCTION

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INTRODUCTION: With age, the microbial balance in the gut becomes disturbed, contributing to systemic inflammation. Pro-inflammatory factors that reach the bloodstream can damage the integrity and function of the blood-brain barrier (BBB), causing inflammation in the central nervous system. This can result in neuronal damage and progressive decline in cognitive function (CF). While the role of gut microbiota in the gut-brain axis is increasingly recognised, the exact contribution of commensal bacteria and their secreted components remains unclear.

AIM(S): To characterise the bacterial extracellular vesicles (BEVs) produced by the commensal bacterium Bifidobacterium animalis subsp. animalis CCDM 366 (Ba366), and to investigate whether Ba366 and its BEVs have a protective effect on the BBB.

METHOD(S): Ba366 was obtained from the Laktoflora Culture Collection (Milcom, Tábor, Czech Republic). Human brain microvascular endothelial cells (HBEC-5i, ATCC) were used as an in vitro BBB model. Cell viability and proliferation were assessed using the Incucyte S3 live-cell analysis platform. Tight junction (TJ) protein expression was evaluated via Western blotting. Cytokine secretion (IL-1β, TNF-α IL-6) was measured using ELISA.

RESULTS: Both Ba366 and its BEVs were non-toxic to HBEC-5i cells and could modulate their proliferation. Western blot analysis revealed significant changes in TJ protein expression, suggesting an influence on BBB permeability. ELISA results showed that Ba366/BEVs selectively stimulated IL-1β secretion, while no significant effect was observed for TNF-α or IL-6.

CONCLUSIONS: Ba366 and its BEVs can modulate BBB cell function by regulating TJ protein expression and selectively producing cytokine secretion. These findings suggest their potential role in maintaining or restoring BBB integrity, particularly under inflammatory conditions.

FINANCIAL SUPPORT: This work was partially supported by the Biocodex Microbiota Foundation.

P2.32. AGING IN AN ARTIFICIAL WORLD: EXPLORING THE RETINAL AND BEHAVIORAL CONSEQUENCES OF LIGHT POLLUTION IN AGING DROSOPHILA MELANOGASTER

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INTRODUCTION: The interplay between aging and environmental factors such as light pollution is critical in understanding retinal health, as they can alter neuronal and glial function. The presynaptic protein Bruchpilot (BRP) is vital for synaptic integrity and neurotransmitter release, and alpha subunit of ATP synthase (αATP) is a component of glial metabolism. It was previously shown that the expression level of BRP changes daily in the photoreceptor terminals, which affects not only photoreception but also coordination between the visual system and clock neurons. On the other hand, αATP expression is rhythmic in epithelial glia in the first optic neuropile, lamina. Aging retina shows many changes, possibly also in daily rhythmicity. Light pollution worsens these effects, potentially accelerating neurodegenerative processes.

AIM(S): We investigated the effects of aging and light pollution on daily changes in the expression of BRP in R1-R6 photoreceptor terminals and αATP in epithelial glia in the lamina, as well as effects on visual processing.

METHOD(S): Flies were kept in normal or dim light at night lighting conditions. 7-, 30-, or 60-day old flies were decapitated, cryosectioned, and immunostained against BRP or αATP; fluorescence intensity from confocal images was analyzed. Optomotor response in 30-day old flies was tested using an apparatus with moving visual stimuli.

RESULTS: Rhythm of BRP expression observed in young flies declines with age, while αATP rhythm changes its pattern. Light pollution caused disruptions in the rhythms observed in the visual system. Optomotor responses also declined with both age and artificial light exposure.

CONCLUSIONS: Aging and light pollution are key elements disrupting the circadian rhythms in the Drosophila visual system. Loss of rhythmic optomotor responses highlights the behavioral consequences of molecular changes detected in this study. These findings provide insight into the cellular and behavioral consequences of clock disruptions, with implications for retinal degeneration.

FINANCIAL SUPPORT: This work was supported by the NCN OPUS grant 2022/47/B/NZ3/00250.

P2.33. EXPLORING RETINAL AGEING IN A LIGHT-POLLUTED ENVIRONMENT ON DROSOPHILA MELANOGASTER AS A MODEL

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INTRODUCTION: Light pollution, the alteration of natural light levels by introducing excessive or misdirected artificial light at night (ALAN), has been regarded as a global environmental concern. ALAN can disrupt the organism's endogenous biological clock, affecting its physiological functions. The retina is the most susceptible to oxidative stress damage due to direct light exposure. The retina has protective mechanisms expressed in a circadian manner, shielding the photoreceptors against external factors such as UV light, particularly at the beginning of the day. The disruption of circadian clock function, which may occur with age and in light-polluted environments, may reduce the effectiveness of the protective mechanism.

AIM(S): Investigating the effects of aging and light pollution on retinal physiology.

METHOD(S): Wild-type flies were maintained under normal light conditions (LD 12: 12; 12 h of light and 12 h of darkness) and under L-DIM conditions (12 h of light and 12 h of dimmed light). Young (7 days) and old (30 days) males were collected at specific time points of the day. Samples were used to check gene expression (qPCR), accumulation of DNA damages (immunostaining against 8-hydroxy-2'-deoxyguanosine), and lipofuscin accumulation.

RESULTS: The aging retina showed changes in the daily pattern of clock and clock-dependent gene expression and increased accumulation of lipofuscin. In addition, flies kept in light-polluted conditions showed higher levels of oxidative DNA damage.

CONCLUSIONS: Aging affects the peripheral clock located in the retina, which can disrupt the functioning of protective mechanisms and, consequently, increase oxidative DNA damage in photoreceptors. Light pollution disrupts the clock mechanism and accelerates the changes observed in the aged retina.

FINANCIAL SUPPORT: NCN; 2022/47/B/NZ3/00250; Project leader: PhD Milena Damulewicz.

P2.34. THE ROLE OF STIM2 IN RETINAL NEURODEGENERATION

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INTRODUCTION: Calcium signaling plays a crucial role in the regulation of neuronal function and survival. STIM2 is a key component of store-operated calcium entry (SOCE), which maintains intracellular calcium homeostasis. Our recent data demonstrate that knockout of stim2 in zebrafish induces a glaucomatous-like retinal neurodegenerative phenotype. Intracellular calcium fluctuations are known to regulate microglial migration, functional polarization, phagocytosis, and cytokine release. Increased intracellular calcium levels are associated with microglial activation, a process implicated in neurodegeneration.

AIM(S): To elucidate the mechanisms by which loss of stim2 contributes to neuronal degeneration in the zebrafish retina.

METHOD(S): Immunohistochemistry, transmission electron microscopy.

RESULTS: In stim2 KO retinas, we observed a ~1.8-fold reduction in the number of GABAergic neurons in the inner nuclear layer and a ~1.3-fold reduction in photoreceptor cell density. Moreover, transmission electron microscopy revealed narrowing of the inner plexiform layer (IPL) and ganglion cell layer (GCL), accompanied by a significant decrease in dendritic density and ganglion cell number. Additionally, in photoreceptors, mitochondrial cristae area was reduced by ~50%, indicating severe impairment of mitochondrial structure and energy metabolism.

CONCLUSIONS: We propose that impaired STIM2-dependent calcium signaling alters microglial behavior, promoting neuroinflammation and contributing to neuronal loss. This model potentially offers a new approach to studying the role of calcium dysregulation and microglia in glaucomatous-like retinal degeneration and may reveal novel therapeutic targets for neurodegenerative diseases.

FINANCIAL SUPPORT: Preludium Grant NCN 2023/49/N/NZ3/02921.

P2.35. PREVENTING COGNITIVE DECLINE THROUGH COGNITIVE ENGAGEMENT: WHAT CAN WE LEARN FROM ADULT LEARNERS

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INTRODUCTION: The prevention of cognitive decline is an urgent challenge in aging societies. This study explored the relationship between lifelong learning and cognitive reserve (CR) in healthy adults. Adult learners represent a unique population for examining the protective effects of lifelong cognitive stimulation. This study presents profiles of healthy adults aged 18-72 engaged in various informa, structured educational activities and investigates how these activities relate to their levels of cognitive reserve.

AIM(S): Investigation how lifelong learning and cognitive reserve affect the delay of cognitive abilities. This is crucial for promoting and organizing preventive strategies.

METHOD(S): Using a cross-sectional, quantitative design, 50 participants completed the Cognitive Reserve Index questionnaire (CRIq).

RESULTS: Results revealed a strong correlation between total CR and educational activities (CRI-Edu): $r(50)=.838$, $p<.001$, indicating that higher engagement in learning is significantly associated with increased cognitive reserve. A linear regression model showed that age significantly predicted CRI-Total ($\beta=0.795$, $p<.001$), while gender did not ($\beta=0.03$, $p=.298$). Although cognitive reserve have not been correlate with spatial navigation in this sample, findings support the theoretical link between cognitive engagement and cognitive resilience. Existing literature consistently shows that higher cognitive reserve is associated with better executive functioning and reduced dementia risk.

CONCLUSIONS: Role of cognitive engagement as a modifiable factor in building cognitive resilience is very important. Promoting formal and informal but structured learning opportunities may help enhance cognitive reserve and delay cognitive decline in aging populations. This study also discusses the societal implications of these results, highlighting the importance of accessible lifelong learning, the role of occupational therapy, and community-based institutions in promoting cognitive health in older adults.

FINANCIAL SUPPORT: This study was a part of Msc Dissertation.

P2.36. AMOTL2 IN THE BRAIN: FROM DEVELOPMENT TO POSTNATAL

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INTRODUCTION: Angiomotins, which include AMOT, AMOTL1, and AMOTL2, are scaffolding proteins mainly studied for their role in regulating the Hippo signaling pathway and cancer. However, the function of Angiomotins in the CNS is widely unknown; AMOT has been reported to regulate dendritic outgrowth and spine formation. However, AMOTL2 functions within the brain remain unexplored.

AIM(S): We aim to elucidate the role of AMOTL2 in neural progenitors and its function in differentiated neurons.

METHOD(S): We generated a conditional knockout (cKO) mouse model using the Nestin-Cre line, enabling early deletion of AMOTL2 in neural progenitors. Histological and molecular analyses were performed on neonatal brains to assess changes in brain morphology and cell populations. In addition, cellular and molecular assays were conducted to explore AMOTL2 function in mature neurons.

RESULTS: Our In situ hybridization (ISH) results indicate that AMOTL2 is expressed in both neural progenitors and differentiated neurons, suggesting that it may serve distinct functions at different stages of neurodevelopment. To investigate its role, we generated a conditional knockout using the Nestin-Cre mouse line, targeting AMOTL2 deletion in neural progenitors that give rise to both neurons and astrocytes. This early deletion resulted in altered brain morphology in newborn mice, along with changes in neural progenitors and differentiated neuronal populations. In parallel, our advanced molecular and cellular studies demonstrated that AMOTL2 may also play important functions in differentiated neurons that can affect brain functioning within specific brain regions and subcircuits

CONCLUSIONS: Collectively, our results point to important AMOTL2 functions in the central nervous system. We are currently employing a combination of molecular, cellular, and behavioral approaches to further elucidate the role of AMOTL2 in the brain.

FINANCIAL SUPPORT: This research was supported by Polish National Science Center grants Opus 2022/45/B/NZ3/03688 and Preludium 2024/53/N/NZ3/03243.

P2.37. COMPARATIVE HISTOLOGICAL ANALYSIS OF CORTICAL DEVELOPMENT IN BTBR AND C57BL/6 MOUSE MODELS AT MID-GESTATION STAGES: IMPLICATIONS FOR NEURODEVELOPMENTAL DISORDERS

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INTRODUCTION: Disruptions in mid-gestational cortical development have been implicated in the etiology of autism spectrum disorder (ASD). The BTBR T+ Itpr3tf/J (BTBR) mouse strain, a widely used ASD model, exhibits behavioral and anatomical abnormalities resembling the human condition, whereas the C57BL/6 (B6) strain serves as a comparison for typical development.

AIM(S): In this study, we investigated early developmental differences between BTBR and B6 fetuses at 12.5 and 15.5 days post coitum (dpc).

METHOD(S): Body weight measurements and somite counts were used to assess systemic growth and developmental staging. Histological analysis with hematoxylin and eosin staining was performed to evaluate cortical architecture at 12.5 and 15.5 dpc. Transcriptomic profiling was conducted on fetal heads and placentas to identify strain-specific gene expression patterns.

RESULTS: BTBR fetuses exhibited consistently lower body weights across pregnancy, indicating systemic growth restriction. Somite counts at 12.5 dpc showed no significant differences, confirming equivalent developmental staging. Histology revealed no differences in cortical thickness at 12.5 dpc. By 15.5 dpc, BTBR cortices displayed a thinner ventricular zone and a thicker intermediate zone compared to B6 controls, suggesting altered neurogenesis and/or neuroprogenitor migration. Transcriptomic profiling showed significant gene expression differences between strains, with greater divergence in placental tissue than fetal tissue.

CONCLUSIONS: BTBR fetuses exhibit early structural and transcriptional alterations during cortical development. The pronounced gene expression differences in the placenta suggest a potential non-neural contributor to neurodevelopmental outcomes. Current efforts to generate BTBR-B6 chimeric mice aim to dissect cell-autonomous versus non-cell-autonomous contributions to neurodevelopment. These findings underscore the importance of early development in shaping long-term neurodevelopmental trajectories.

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P2.38. EARLY-LIFE NUTRITIONAL STRESS MODULATES LEPTIN-INDUCED AXONAL GROWTH IN A SEX-DEPENDENT MANNER

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INTRODUCTION: Neurons of the arcuate nucleus (ARC) are central integrators of metabolic signals within hypothalamus, with leptin serving as a crucial modulatory hormone. Among these, kisspeptin-expressing neurons play an essential role in regulating the reproductive axis, and their development is particularly sensitive to metabolic status.

AIM(S): To examine how early-life undernutrition impacts this system, we analyzed leptin-induced axonal growth in ARC explants from suckling mice, with a focus on sex-specific responses.

METHOD(S): We crossed two transgenic mouse strains to generate offspring expressing tdTomato fluorescence in kisspeptin neurons using the Cre-loxP system. Newborn pups were assigned to either control group (CON; dams fed ad libitum during lactation) or lactation undernutrition group (LUN; dams received 50% of the control diet). On postnatal day 8, ARC explants were microdissected and cultured separately by sex. After 24 hours in vitro, explants were treated with leptin or vehicle. Following another 24 hours, tissues were fixed, immunostained for neurofilaments and imaged using fluorescence microscopy. Axon length was quantified with Zeiss ZEN Blue Software.

RESULTS: Leptin promoted axonal growth in ARC explants from CON females, an effect not observed in males. Notably, in LUN females, leptin failed to stimulate axon outgrowth. However, when analysis restricted to kisspeptin neurons-specific axons, which were markedly shorter than those of the general ARC population, the leptin response was preserved in LUN females but completely absent in LUN males.

CONCLUSIONS: To our knowledge, this is the first study to directly quantify axon growth in kisspeptin neurons and to reveal sex-dependent differences in this process. Our findings highlight the existence of sex-specific sensitivity in the developing hypothalamus to early-life nutritional stress, with potential long-term consequences for reproductive function and the integration of metabolic and reproductive pathways.

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P2.39. REISSNER FIBER FORMATION REQUIRES MULTIPLE DEVELOPMENTAL INPUTS INCLUDING POTASSIUM CHANNELS AND GRAVITY

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INTRODUCTION: Reissner fiber is a rope-like structure composed of microfilaments that originate from the midline floor plate and the anterior roof plate-derived subcommissural organ (SCO). Reissner fiber extends through the brain ventricular system (BVS) and the central canal to the end of the spinal cord. Reissner fiber is found in vertebrates with horizontal body posture. Reissner fiber consists of the giant matricellular protein Scospondin and several auxiliary polypeptides.

AIM(S): We aimed to understand the developmental mechanisms regulating the formation of Reissner fiber.

METHOD(S): We used a combination of the in vitro and vivo immunohistochemistry, light-sheet microscopy, and genetic and toxicological analyses to study Reissner fiber development in zebrafish.

RESULTS: The Reissner fiber develops through two mechanisms. First, the posterior Reissner fiber forms due to secretion from the apical surface of the floor plate. This fiber remains attached to the anterior floor plate (flexural organ); though in the hindbrain it begins to detach from the floor plate soon after formation. Second, the anterior Reissner fiber forms in the cerebral aqueduct cavity through secretion from the SCO. It then attaches to the flexural organ, where it fuses with the posterior fiber. Later, the SCO elongates posterior ward along the fiber trajectory through mechanoelastic stretching. Analysis of mutants affecting the two subunits of the voltage-gated potassium channel Kv2.1 revealed that Kv2.1 regulates BVS and Reissner fiber development. Toxicological analyses demonstrated that Reissner fiber formation depends on Hedgehog and Wnt/β-catenin signaling pathways, as well as cholesterol acting upstream of Kv2.1. Maintaining the embryo in abnormal orientation results in Reissner fiber and SCO deformation.

CONCLUSIONS: The development of the Reissner fiber takes place in two stages by the floor plate and SCO. It depends on multiple internal (developmental) and external (gravity) inputs. Reissner fiber shapes SCO and flexural organ.

FINANCIAL SUPPORT: NCN, OPUS 2020/39/B/NZ3/02729.

P2.40. IDENTIFICATION OF THE MECHANISMS AND BIOMARKERS OF MCOPS12 TO PROPOSE TREATMENT STRATEGIES

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INTRODUCTION: Microphthalmia syndromic 12 (MCOPS12) is a rare neurologic disease caused by the point mutations of Retinoic Acid Receptor β (RARβ). It affects striatum, eyes and selected internal organs. Regulation of the retinoic acid (RA) pathway is crucial for their development. The disease is linked to mutations in RARβ and is caused by its de novo mutation. In collaboration with clinicians, team has generated and validated mouse models for MCOPS12. Mice like patients display motor and cognitive deficits . We found that MCOPS12 mouse model displays defect in cholesterol brain metabolism. The clearance of excess cholesterol from the brain involves enzyme cyp46A1. It is responsible for catalyzing reaction of cholesterol is conversion into 24-hydroxycholesterol.

AIM(S): Identification of the mechanisms and diagnostic markers in MCOPS12 to orient potential treatment strategies to deliver and help scientific community as well as affected patients to broaden the understanding of the matter.

METHOD(S): Primary neuronal cultures were established from Wt and RARb mice carrying null mutation (KO) or disease causing R387C point mutation, cultured for 7 days and used for immunofluorescenct analyses of protein expression (IF) combined with determination of cholesterol localization.

RESULTS: IF analyses showed difference in cyp46A1 expression between mutants and WT as well as cholesterol intracellular distribution. Lysosomal changes were also observed and potentially associated with abnormal cholesterol distribution in mutant mice. This was associated with altered acid alpha-glucosidase (GAA) amount that were also observed to be affected in mutant mice.

CONCLUSIONS: Investigations in this area can put more light into topic of rare diseases expanding understanding of the processes behind them and help finding treatment strategies.

FINANCIAL SUPPORT: Wrocław University of Science and Technology PhD scholarship BGF – French government scholarship Cure MCOPS12 foundation

P2.41. CONTRIBUTION OF RXRG-DEPENDENT OLIGODENDROCYTE TO AGING- AND DISEASE-ASSOCIATED OLIGODENDROGENESIS

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INTRODUCTION: Overcoming remyelination failure in multiple sclerosis (MS) remains a major challenge in the development of effective therapies. Successful remyelination requires the differentiation of oligodendrocyte precursor cells (OPCs), a process that is impaired in MS. Retinoid X receptor gamma (RXRγ), a nuclear receptor known to promote OPC differentiation, is downregulated in MS and this reduction correlates with remyelination failure. However, the precise mechanisms by which RXRγ regulates remyelination, and its potential role in developmental oligodendrogenesis, are still unclear.

AIM(S): We aim to uncover RXRγ role during oligodendrogenesis and its mechanism of action.

METHOD(S): For analyses of RXRg-dependent oligodendrocyte lineage we used new mouse reporter line suitable for cell-fate tracing analyses. To determine functional relevance of RXRg expression in oligodedrogenesis we studied effects of RXRg loss-of-function in RXRg-/- mice using transcriptomic analyses of primary OPCs cultured in vitro or single-nuclei RNA-sequencing (snRNAseq) of corpus callosum samples. Selection of deregulated genes was confirmed using immunofluorescent analyses in mouse brain.

RESULTS: We found that RXRg does not contribute to any major events during pre- or postnatal oligodendrogenesis, but is expressed during regenerative oligodendrogensis associated with aging, or induced in the young adult mouse brain following chemical demyelination. In this context, wild-type, RXRγ-positive OPCs differentiate into mature oligodendrocytes and contribute to remyelination process. In contrast, RXRg-/- exhibit impaired remyelination. Using snRNA-seq we identified a unique molecular signature of RXRg-dependent oligodendrocyte lineage which may directly underlie hypomyelination observed in RXRg-/- brain.

CONCLUSIONS: RXRg is new selective marker and modulator of aging- or disease-associated oligodendrogenesis.

FINANCIAL SUPPORT: France SEP.

P2.42. A NOVEL CROSS-SPECIES P-TAU217 IMMUNOASSAY FOR INVESTIGATING ANIMAL MODELS OF ALZHEIMER'S DISEASE

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INTRODUCTION: The measurement of phosphorylated tau (pTau) in biofluids, particularly at position 217 (pTau217), has proven to be a valuable diagnostic and prognostic marker in Alzheimer's disease (AD). However, due to tau variability across species existing immunoassays lack compatibility with mouse models, posing a challenge for studying these markers in preclinical research.

AIM(S): Development and optimization of a novel cross-species p-tau217 immunoassay to investigate animal models of AD.

METHOD(S): A novel pTau217 immunoassay was developed and validated on the Simoa platform with plasma samples from three distinct mouse models containing either endogenous or transgenic human tau (APP23, P301S and WT) and human plasma samples for cross-species validation. Optimization for sensitivity, specificity, and reproducibility included different antibodies concentration tests, limit of quantification, and buffer adjustments to diminish plasma matrix interference.

RESULTS: The sequence alignment guided the selection of antibodies targeting conserved epitopes, ensuring cross-species applicability. The novel pTau217 immunoassay, based on Simoa technology, demonstrated good performance in detecting pTau in brain extracts and plasma across all three mouse models containing endogenous mouse tau and transgenic human tau. It also proved to detect human plasma pTau217. The assay exhibited high sensitivity, achieving reliable detection of pTau even in plasma samples with low abundant protein concentrations and matrix interference effect, and showed robust specificity to pTau217.

CONCLUSIONS: We present the validation of the first Simoa-based pTau217 immunoassay designed to mea-

sure p-tau across species (mouse and human), whether in plasma samples with genuine mouse tau sequence or transgenic human tau sequence. Further development will focus on linearity tests and correlation with neuropathologic changes. Our findings have broad implications for biomarkers research and help bridge the gap between human and animal studies in AD research.

FINANCIAL SUPPORT: Fondation Recherche Alzheimer Paris Brain Institute (PBI).

P2.43. MITOCHONDRIAL BIOGENESIS ON PAUSE: MTDNA DOWNREGULATION DESPITE PGC1A UPREGULATION IN EARLY HUMAN EBS

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INTRODUCTION: Mitochondrial remodeling supports the metabolic and transcriptional shifts required for stem cell differentiation. A key component of this process is mitochondrial biogenesis, which governs mtDNA replication and mitochondrial expansion. However, how biogenesis is regulated during early embryoid body (EB) formation remains poorly characterized.

AIM(S): This study aimed to evaluate mitochondrial DNA (mtDNA) content and the expression of mitochondrial biogenesis regulators in early human EBs.

METHOD(S): Unguided 14-day embryoid bodies were generated from a human induced pluripotent stem cell (iPSC) line using only Essential 6 media. mtDNA copy number was measured by quantitative PCR using ND1/HBB and ND5/SERPINA1 ratios. Expression of PPARGC1A, NRF1, and TFAM was assessed by reverse transcription qPCR (RT-qPCR) and normalized to GAPDH. Three biological replicates were analyzed. Data were tested for normality and compared using unpaired, two-tailed t-tests in GraphPad Prism.

RESULTS: Compared to the iPSC line, embryoid bodies showed a significant decrease in mtDNA copy number. This reduction was accompanied by increased expression of PPARGC1A, stable levels of NRF1, and significantly reduced TFAM expression, suggesting a potential disconnect between mitochondrial biogenesis initiation and mtDNA maintenance.

CONCLUSIONS: These findings suggest a transcriptional uncoupling within the mitochondrial biogenesis pathway, with upstream activation occurring in the absence of downstream mtDNA maintenance. This may reflect a regulatory pause in mitochondrial transcription during early lineage transition.

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P2.44. MODELING NEURONAL AGING IN HIPSC-DERIVED DOPAMINERGIC NEURONS: D-GALACTOSE-INDUCED EPIGENETIC DEREGULATION, MITOCHONDRIAL DYSFUNCTION, AND SENESCENCE-ASSOCIATED PHENOTYPES

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INTRODUCTION: Aging is characterized by progressive molecular and epigenetic changes, including heterochromatin disorganization and impaired stress responses, which contribute to the onset of neurodegenerative disorders such as Parkinson's disease (PD). Epigenetic deregulation, in particular, has emerged as a central mechanism underlying age-related neuronal dysfunction. Experimental models that recapitulate these changes are essential for understanding the mechanisms driving neurodegeneration.

AIM(S): The study aimed to establish a cellular model of neuronal aging using D-galactose and to assess age-related molecular and epigenetic alterations in dopaminergic neurons derived from hiPSCs

METHOD(S): Dopaminergic neurons were differentiated from human-induced pluripotent stem cells (hiPSCs) and exposed to D-galactose (15 mg/mL) for five days. Epigenetic changes were analyzed using immunofluorescent staining for heterochromatin-associated proteins HP1γ and H3K9me3. Evaluations included cellular senescence, viability, oxidative stress levels, mitochondrial performance, and transcriptional activity.

RESULTS: Exposure to D-galactose induced a marked reduction in chromatin compaction, as evidenced by significantly lower levels of HP1γ and H3K9me3. Neurons displayed heightened oxidative stress, diminished mitochondrial functionality, and suppressed expression of mitochondrial biogenesis regulators including PGC1a and NRF1. The senescence-associated gene P16 showed increased expression. Additionally, dopaminergic neuronal markers TH and MAP2 expression declined significantly, accompanied by upregulation of the pro-apoptotic gene BAX.

CONCLUSIONS: This model demonstrates that D-galactose induces an aging-like phenotype in dopaminergic neurons, marked by epigenetic disorganization, mitochondrial dysfunction, loss of dopaminergic markers, and senescence-associated gene expression.

FINANCIAL SUPPORT: The study was supported by Mossakowski Medical Research Institute, Polish Academy of Sciences – Research Fund grant no. FBW 032/2022.

P2.45. BRINGING MARMOSET TO EBRAINS

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INTRODUCTION: EBRAINS is one of the most potent platforms for advancing neuroscience research. While it provides extensive support for rodent (predominantly mouse) and human-oriented datasets, it currently supports only one non-human primate (NHP) species despite the critical role of NHPs in bridging the translational gap between rodent and human studies.

AIM(S): To address this limitation, we propose to expand the EBRAINS platform by incorporating a new atlas of the marmoset (Callithrix jacchus) cerebral cortex, which we will call Marmoset@EBRAINS. This atlas will be accompanied by diverse datasets such as neuronal distribution and cellular-level connectivity registered to this new reference framework.

METHOD(S): The proposed atlas will be derived from the Nencki-Monash marmoset brain template (NM template), a gender-balanced, morphological average of 20 young adult marmosets. Based on Nissl histology, the template combines the detailed cytoarchitectural information of histology-based atlases with the isotropic resolution and probabilistic analyses typical of MR-based templates. We will then complement the new framework with multimodal datasets, including comprehensive maps of neuronal distribution in the cortex and results from 143 experiments investigating cortical area connections using fluorescent tracers. Additionally, we will demonstrate how EBRAINS users can map their datasets onto the Marmoset@EBRAINS atlas using existing EBRAINS digital atlasing tools.

CONCLUSIONS: This project will lay the groundwork for the broad integration of the marmoset as a model species within EBRAINS. The project will directly benefit the EBRAINS initiative by enabling more extensive cross-species analyses and encouraging other marmoset research groups to integrate their datasets with the new framework, thereby expanding the user base. It also represents a significant step towards generalising the available atlasing tools, enhancing the platform's versatility.

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P2.46. MAPPING CELLULAR DIVERSITY AND SPATIAL ARCHITECTURE USING CUTTING-EDGE TRANSCRIPTOMIC TECHNOLOGIES

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INTRODUCTION: Advanced transcriptomic technologies are essential for unraveling gene expression complexity and offer powerful tools for studying biological systems and disease mechanisms. These methods are increasingly central to both basic and translational neuroscience.

AIM(S): This poster highlights how bulk, single-cell, and spatial transcriptomic technologies have been applied in preclinical and disease-modeling studies supported by GeneCore, an academic facility specializing in advanced transcriptomics. We aim to demonstrate how these approaches advance understanding of disease mechanisms and therapeutic strategies.

METHOD(S): Bulk RNA sequencing was used to study responses to delayed C3a treatment in a photothrombotic mouse stroke model. Single-cell RNA sequencing characterized early pathology in in vitro models of Alexander disease. Spatial transcriptomics mapped glial responses in a permanent ischemia model. We also summarize efforts to standardize small RNA analysis protocols.

RESULTS: Bulk transcriptomic profiling revealed that delayed C3a treatment suppresses astrocyte inflammatory responses in the peri-infarct cortex and upregulates genes involved in synaptic function. Sin-

gle-cell analysis identified aberrant neurodevelopment in human iPSC-derived models of Alexander disease, suggesting a potential novel disease mechanism. Spatial transcriptomics captured region-specific and time-dependent glial responses, offering insights into molecular and cellular dynamics during recovery. Although technically demanding, small RNA profiling is a feasible approach for system-level or targeted characterization of regulatory miRNA networks.

CONCLUSIONS: The integration of bulk, single-cell, and spatial transcriptomic approaches enables comprehensive analysis of gene expression programs, cellular diversity, and tissue organization. Together, these technologies provide actionable insights into neurological disease mechanisms and support the development of targeted therapeutic strategies.

P2.47. TOPOGRAPHIC MAPPING OF VISUAL INPUT TO DOPAMINERGIC MIDBRAIN STRUCTURES IN THE RAT

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INTRODUCTION: The superior colliculus (SC) is a midbrain structure essential for integrating visual information and initiating orienting behavior. Beyond its classical role in sensorimotor coordination, recent findings suggest that the SC modulates reward-related dopaminergic circuits. While lateralized SC projections to midbrain dopaminergic nuclei have been described in rats, the spatial organization of this influence remains largely unexplored.

AIM(S): This study aimed to develop a robust and spatially precise in vivo setup that enables the functional mapping of SC-driven visual input onto dopaminergic neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA).

METHOD(S): We designed a custom 3D-printed head-surrounding chamber for use with anesthetised, stereotaxically mounted Sprague-Dawley rats. The chamber houses an 8×32 LED matrix programmed via Arduino to emit 10-ms light flashes at defined spatial coordinates, allowing targeted stimulation of specific retinal regions. The system is optimized for concurrent extracellular single-unit recordings in SNc and VTA using high-impedance glass electrodes.

RESULTS: We successfully developed and validated a stable and reproducible experimental setup suitable for precise visuo-electrophysiological studies in the rat midbrain. The system enables fine control over the spatial parameters of visual stimulation and allows for stable single-neuron recordings. Pilot tests suggest the potential to resolve topographic patterns of SC-driven visual input, which will be systematically tested in the upcoming recording experiments.

CONCLUSIONS: Our setup enables high-resolution functional mapping of visual input to midbrain dopaminergic circuits in vivo. It lays the foundation for uncovering whether and how the SC exerts spatially organized control over reward-related structures, with relevance for understanding subcortical sensory-motivational integration.

P2.48. A BIOORTHOGONAL PUTRESCINE PROBE TO TRACK POLYAMINE UPTAKE AND FLUX UNDER NEUROTOXIC STRESS

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INTRODUCTION: Putrescine (Put), like other polyamines, participates in numerous cellular processes in the brain, including neurogenesis, synaptic transmission, plasticity, and response to neurotoxic stress. The cellular specificity of polyamine transport and its stress-induced dynamics remain poorly understood, largely due to the lack of suitable analytical tools.

AIM(S): We aimed to investigate the cellular specificity of Put uptake by comparing neurons and astrocytes. Additionally, we aimed to compare Put accumulation across hippocampal subregions and examine changes in its distribution under hypoxic and excitotoxic stress.

METHOD(S): We employed both an in vitro (mixed cortical neuron/astrocyte primary mouse cultures) and ex vivo (acute mouse hippocampal slices) models. A propargyl-modified Put analogue (TvS-Put) served as a bioorthogonal probe that, upon cellular uptake, was fluorescently tagged via a click chemistry reaction. Hypoxia and excitotoxicity were pharmacologically modelled by exposing slices to CoCl2 and NMDA, respectively.

RESULTS: TvS-Put accumulation in cells was inhibited by non-modified Put and specific polyamine transporter inhibitor demonstrating the probe specificity. The probe was preferentially taken up by neurons over astrocytes in both culture and slice models. In neurons, TvS-Put accumulated primarily in nuclei, followed by somata. In hippocampal slices, the probe accumulated more in CA1 than CA2/3; however this pattern was altered following $CoCl_2$ or NMDA treatment, with TvS-Put accumulating equally in CA1 and CA2/3, indicating region-specific regulation of Put transport dynamics in response to neurotoxic stress.

CONCLUSIONS: Our findings demonstrate that TvS-Put is a reliable tool for investigating Put transport and distribution in brain cells. This approach opens new avenues for exploring the role of polyamines in the physiology and pathology of the central nervous system.

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P2.49. A RAPID IMMUNOFLUORESCENCE METHOD COMPATIBLE WITH FUNCTIONAL ASSAYS IN ACUTE AND ORGANOTYPIC SLICES

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INTRODUCTION: Acute and organotypic slices are a powerful tool for short- and long-term experimental manipulations. They support a broad range of imaging-based functional assays using genetically-encoded or chemical fluorescent probes to study cell injury, calcium fluxes, membrane transport and more. Post-assay immunofluorescence can reveal spatially resolved protein expression, providing complementary insight into additional cellular processes and tissue structure. However, properties of acute and organotypic slices differ from fixed brain, making regular immunofluorescence protocols incompatible.

AIM(S): We aimed to develop a rapid immunofluorescence protocol for both slice types, enabling detection of selected proteins following imaging-based functional assays for direct co-analysis or post-imaging comparisons.

METHOD(S): Acute hippocampal slices were obtained from adult mice. Organotypic slices were cultured from hippocampi of rat or mice pups using standard protocols. Slices were subjected to experimental treatment and functional assays, followed by testing of fixation and immunostaining conditions to find parameters effective for both types with minimal processing time.

RESULTS: The method allows precise immunofluorescent labeling of brain regions (e.g., hippocampal CA fields) and cell types (e.g., astrocytes, neurons, CA2 pyramidal neurons) as well as detection of condition-dependent changes in expression of proteins involved in defined cell pathways (e.g. mitochondrial calcium uptake or caspase activation). The procedure takes ~4-5 hours after overnight fixation and is compatible with prior functional assays (e.g. cell labeling, neuronal injury or polyamine uptake).

CONCLUSIONS: This method combines speed, structure preservation and compatibility with functional assays, supporting multi-dimensional analyses in basic and translational neurobiology.

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P2.50. OVERCOMING LIMITATIONS OF OPTOGENETICS: CAN WE AMPLIFY THE SIGNAL?

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INTRODUCTION: Vision is one of the most vital senses, playing a crucial role in how individuals interpret and interact with their environment. However, with the increasing prevalence of environmental stressors, aging population, and lifestyle-related risk factors, eye-related diseases have expanded dramatically over recent decades. Therefore, the scientific community has devoted considerable effort to developing strategies aimed at preserving or restoring visual function. Yet, despite significant advancements in fields such as gene therapy, retinal prosthetics, and optogenetics, no approach has succeeded in fully reversing the damage. Thus, there is an urgent need for innovative and more efficient solution.

AIM(S): The aim of the study is to amplify a signal after restoring initial photosensitivity. This approach involves the introduction of light-sensitive channels to enable the response to various light stimuli. However, introducing photosensitivity alone may not be sufficient to restore functional vision. Because of that, our work focuses on identifying a suitable signal amplifier that can boost the light-induced response.

METHOD(S): The initial screening of potential amplifying candidates was conducted using in vitro patch-clamp technique under the voltage clamp conditions, allowing precise evaluation of channel activity and response dynamics.

RESULTS: Through voltage-clamp recordings, we identified a promising candidate capable of enhancing light-induced signals at the cellular level.

CONCLUSIONS: The obtained results of in vitro experiments proved that we are able to amplify the response to the light stimulation by combining two approaches – introduction of a light-sensitive channel and an amplifier. The selected candidate will be further analyzed during in vivo experiments.

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P2.51. A LOW-COMPUTATIONAL PIPELINE FOR POST-DEEPLABCUT ANALYSIS OF OPEN FIELD TEST DATA USING CENTROID-BASED SPATIAL METRICS

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INTRODUCTION: The Open Field Test (OFT) is widely accepted to assess exploratory behavior, locomotor activity, and anxiety traits in rodents. Healthy rodents exhibit strong thigmotaxis. Hence, time spent and entries into central (aversive) versus peripheral zones are commonly used readouts of anxiety traits. While markerless pose estimation tools such as DeepLabCut (DLC) have greatly improved behavioral tracking, there is a lack of accessible, standardized post-DLC pipeline. This limits reproducibility across laboratories and hinders broader application.

AIM(S): To develop a reproducible, low-computational pipeline for spatial analysis of OFT data, using centroid-based metrics derived from DLC outputs, and to evaluate its applicability in disease models.

METHOD(S): We developed a Python-based post-processing pipeline in Jupyter Notebook that calculates the dynamic centroid of tracked body parts. Using interactive rectangular zone selection, we segmented the open field arena into central and peripheral regions and quantified time spent in each. The approach is tolerant to pixel-level noise introduced by shorter DLC training on limited hardware, making it scalable and time-efficient.

RESULTS: The centroid-based approach successfully tracked exploratory patterns and distinguished between central and peripheral zone occupancy, ignoring pixel fluctuations. Time spent in the center versus periphery was automatically computed per animal. The system allowed for rapid adjustment of zone boundaries and could be adapted for other spatial layouts.

CONCLUSIONS: This pipeline provides an accessible and robust framework for analyzing OFT data post-DLC, requiring minimal computational resources and no proprietary software. It enhances reproducibility and allows broader application of DLC-based behavioral assays.

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P2.52. FUNCTIONAL INTRODUCTION OF RABIES VIRUS TO HEALTHY AND DEGENERATED RETINA

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INTRODUCTION: The Rabies virus (RV) is a (-)RNA rhabdovirus, known for its ability to infect neurons via retrograde transport. In neurons infected with a modified RV, we observed high expression of protein-coding genes. However, there is currently no definitive evidence demonstrating the effectiveness of the G-deleted rabies virus (RVΔG) in infecting retinal cells.

AIM(S): We tested whether RVΔG can infect various types of retinal cells in the healthy and the degenerated retinas, and whether there are differences between the infection of cells in those types of retinas.

METHOD(S): We injected RVΔG carrying a fluorescent protein into the eyes of wild-type and RhoP23H/P23H mice via subretinal delivery. Infected cell types were identified through immunostained retinal whole mounts and cross-sections. Functional vision reactivation in degenerated retinas was assessed using optogenetic recordings after injections of RVΔG carrying blue- and yellow-light-sensitive opsins.

RESULTS: We found that RVΔG is capable of infecting a broad spectrum of retinal cell types, including RGCs. Infection rates were comparable between healthy and degenerated retinas, with no significant differences observed. In optogenetic recordings, light-evoked responses were detected in a 3-month-old RhoP23H/P23H mouse 14–16 days post-infection, indicating functional reactivation of the degenerated retina.

CONCLUSIONS: Our findings suggest that RVΔG is an effective tool for delivering genetic cargo into retinal cells, offering valuable insights into retinal connectivity. Additionally, it shows strong potential for targeting and delivering cargo to cells that are retrogradely connected within the retina.

FINANCIAL SUPPORT: Funded by the National Science Centre, Poland, under project no. 2019/32/E/NZ5/00434 within Sonata bis 9 call and project no. 2020/39/D/NZ4/01881 within Sonata 16 call. The International Centre for Translational Eye Research (FENG.02.01-IP.05-T005/23) project is carried out within the International Research Agendas programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

P2.53. SEARCHING FOR THE PARAMETERS OF NEURON-SPECIFIC EXPRESSION OF AAV VECTORS IN THE MOUSE HYPOTHALAMUS

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INTRODUCTION: Obesity is a serious disease and a major risk factor for cardiovascular conditions, type 2 diabetes, and cancer. According to WHO, around 3.5 billion people worldwide are affected by overweight and obesity. Food intake and energy balance are regulated by anorexigenic POMC and orexigenic AgRP neurons located in the arcuate nucleus (Arc) of the hypothalamus. Their activity is modulated by peripheral signals such as leptin, insulin, and ghrelin, as well as by microRNAs that regulate gene expression. AgRP neurons, involved in food intake, are likely candidates and potential therapeutic targets. Adeno-associated viral (AAV) vectors are a promising tool for their precise manipulation. Careful selection of AAV capsid, ITRs, and gene regulatory elements may ensure specificity and efficiency.

AIM(S): We aimed to evaluate the transduction efficiency and specificity of various AAV capsid serotypes and ITR sequences in hypothalamic cells of C57Bl/6 mice, focusing on their ability to deliver Cre recombinase to specific neuronal populations, with particular emphasis on the Arc and AgRP neurons.

METHOD(S): We produce AAV vectors via triple transfection in a helper-free system. These vectors, incorporating various combinations of natural and synthetic capsids and ITR sequences, carry Cre recombinase. The vectors are stereotactically injected into the Arc region of C57Bl/6 mice. Two weeks post-injection, expression patterns are assessed through immunohistochemistry, focusing on transduced regions and specific cell types.

RESULTS: Results showed that different AAV configurations yield diverse expression patterns in the Arc region of C57Bl/6 mice.

CONCLUSIONS: Understanding the properties of individual components of AAV vectors can contribute to the development of highly precise tools for genetic manipulation, even of selected cell populations, which can aid in advancing gene therapies for complex diseases, enhancing the specificity and safety of treatments, and enabling personalized medicine approaches.

FINANCIAL SUPPORT: This work was funded by Łukasiewicz Research Network – PORT, Polish Center for Technology Development (statutory funds) and by National Science Centre (OPUS) grant 2019/35/B/NZ4/02831.

P3.01. INVESTIGATING THE ROLE OF A VCP/P97 COFACTOR SVIP IN MODULATING HUNTINGTON'S DISEASE PHENOTYPES

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INTRODUCTION: Protein quality control (PQC) is a vital cellular system often impaired in neurodegenerative diseases such as Huntington's disease (HD). Valosin-containing protein (VCP/p97) is a central PQC regulator and its activity is shaped by cofactors that guide its function. HD is caused by polyglutamine expansion in the huntingtin (HTT) protein, leading to toxic aggregation and selective loss of medium spiny neurons (MSNs). While mutant HTT toxicity is well described, the role of VCP/p97 regulation in disease progression remains unclear.

AIM(S): We aim to determine how a selected VCP/p97 cofactor – small VCP-interacting protein (SVIP), identified from HD omics data, influences disease-relevant phenotypes.

METHOD(S): In the study we used a human model of HD based on direct conversion of patient fibroblasts into medium spiny neurons (miR-HD-MSNs), that by omitting the pluripotency stage preserves aging signatures of donor cells.

RESULTS: In miR-HD-MSNs, we assessed the expression level of SVIP and examined the effect of its manipulation on disease-relevant phenotypes, including HTT protein accumulation and cell survival. Preliminary data suggest that SVIP is enriched in neurons and that modulating its expression in HD-MSNs alters autophagic flux, potentially contributing to its functional effects.

CONCLUSIONS: Our study provides initial evidence that the VCP/p97 cofactor SVIP plays a modulatory role in Huntington's disease pathology. Its enriched expression in neurons and impact on autophagic flux suggest that SVIP contributes to the regulation of proteostasis in HD-affected neurons. These findings support further investigation into SVIP as a candidate for targeting PQC dysfunction in Huntington's disease.

FINANCIAL SUPPORT: The project is financed from the Sonata Bis 11 Grant (2021/42/E/NZ3/00439) – National Science Centre Poland.

P3.02. ASSESSMENT OF MITOPHAGY IN HUMAN SUBPALLIAL ORGANOIDS ENRICHED WITH GABAERGIC NEURONS IN DRAVET SYNDROME

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INTRODUCTION: Mitochondria are essential for cellular energy and homeostasis, with mitophagy (a specialized form of autophagy) playing a key role in removing damaged mitochondria. Recent studies suggest mitochondrial dysfunction may contribute to neurodevelopmental disorders. Dravet Syndrome (DRVT), a severe developmental and epileptic encephalopathy associated with SCN1A mutations, has unclear underlying mechanisms, but emerging evidence points to possible mitophagy dysregulation.

AIM(S): The aim of this study was to assess the mitophagy in a human 3D ventral forebrain organoid model enriched for GABAergic neurons derived from Dravet Syndrome patients.

METHOD(S): Two induced pluripotent stem cell (iPSC) lines derived from DRTV patients with SCN1A mutations and healthy control iPSC line were used for derivation of ventral (subpalial) forebrain organoids enriched for GABAergic neurons using dual SMAD inhibition, SHH agonist and WNT pathway inhibitor, and sequential treatment with EGF, bFGF, BDNF and NT3. Organoids were collected on days 60, 75, and 100 for immunocytochemistry and on 100 day for gene expression analysis.

RESULTS: Ventralisation and GABAergic neuron enrichment were confirmed by expression of markers including GABA, EOMES, DLX2, GAD67, and somatostatin. Mitophagy was detected by the presence of BNIP3 and LC3B-positive cells at day 100. The downregulation of mitophagy-related genes (PINK1, PARK2, BNIP3) was detected in DRVT organoids compared to control. Additionally, altered expression of mitochondrial biogenesis genes, such as NRF1, PPARGC1, TFAM was observed in DRVT organoids.

CONCLUSIONS: Our findings suggest that mitophagy and mitochondrial biogenesis are impaired in Dravet Syndrome ventral forebrain organoids, supporting the hypothesis that mitochondrial dysregulation may contribute to the pathogenesis of DRVT. These results highlight mitophagy as a potential therapeutic target for further investigation.

FINANCIAL SUPPORT: Mossakowski Medical Research Institute internal grant FBW-035 and Statutory Funds.

P3.03. INTEGRATING COMPUTATIONAL AND EXPERIMENTAL APPROACHES TO REVEAL TAURINE'S NEUROPROTECTIVE ROLE IN RETINAL ISCHEMIA

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INTRODUCTION: Taurine and its natural derivatives show therapeutic potential in various diseases, but their role in CNS ischemia/reperfusion injury (IRI), including retinal IRI (RIRI), is not fully understood.

AIM(S): This study aimed to integrate computational and in vivo evidence to evaluate the neuroprotective effects of taurine and its derivatives in CNS IRI, using RIRI as a model.

METHOD(S): Network pharmacology and molecular docking were used to identify taurine-related targets and assess binding affinities of taurine and its derivatives (N-acetyltaurine, N-chlorotaurine, N-bromotaurine, glutaurine, taurocholic acid, and tauroursodeoxycholic acid) to key proteins involved in brain IRI. To validate these findings, RIRI was induced in adult male Wistar rats by raising intraocular pressure to 110mmHg for 60 minutes; the contralateral eye served as control. Rats received 0.2M taurine in drinking water or remained untreated for 2 weeks before injury and during 24 hours or 7 days of reperfusion. Retinal gliosis, inflammation, and apoptosis were assessed via immunohistochemistry and Western blotting.

RESULTS: Network pharmacology identified 418 taurine-related targets in brain IRI, enriched in apoptosis, immune response, metabolism, and PI3K-Akt pathways. Docking simulations showed strong binding of taurine and its derivatives to key apoptotic proteins (APAF1, cytochrome c, AKT1, SHP2). In vivo, taurine reduced RIRI-induced astrocyte branching and upregulation of GFAP, IL-1β, TNF-α, and cleaved caspase-3, with no effects in sham eyes, indicating injury-specific action.

CONCLUSIONS: Taurine shows neuroprotective potential in RIRI by dampening reactive gliosis, inflammatory cytokine expression, and neuronal apoptosis. Although further validation of the molecular targets of taurine and its derivatives is needed, this study highlights the strength of combining in silico and in vivo approaches to link molecular interactions with neuroprotective effects, underscoring the value of integrated strategies to advance CNS therapeutics.

FINANCIAL SUPPORT: This research was supported by internal funding. No external financial support was received.

P3.05. MUSCLEBLIND-LIKE RNA BINDING PROTEIN TRAFFICKING DYNAMICS ARE REGULATED BY MOLECULAR INTERACTIONS WITH EARLY AND RECYCLING ENDOSOMES

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INTRODUCTION: Transport of mRNAs and localized translation are crucial for neurodevelopment and function. Recent findings revealed a role of endosomes in RNA transport and localized translation in conjunction with RNA binding proteins (RBPs). Muscleblind-like (MBNL) proteins are RBPs that regulate splicing and mRNA localization. MBNLs are depleted from the cytoplasm in myotonic dystrophy type 1 (DM1) by sequestration on the expanded CTG repeats in the 3' UTR of dystrophia myotonica protein kinase (DMPK) transcripts. We have shown a role for MBNL-kinesin interactions in mRNA localization although the role of membrane association is not understood.

AIM(S): We hypothesized that the unstructured carboxy terminus (C-term) of MBNL1 regulates endomembrane attachment necessary for mRNA localization.

METHOD(S): Fixed and live cell imaging in cell lines and mouse neurons. Overexpression of EGFP-MBNL1 with or without C-term, followed with immunofluorescence (IF) for endo- and lysosome markers (Rab5, Rab7, Rab11, Lamp1) in n2a cells. Widefield live imaging of EGFP-MBNL1 with endo- and lysosomes (Rab5, Rab11, Rab7 and Lamp1), then MBNL1 and Rab5/Rab11 endosomes with the expression of mutants (dominant negative Rab5 S43N, MBNL1 with C-term deletion). Superresolution (STED) live imaging with MBNL1 RNA binding deficient mutant fused to MCP-Halo, 24xMS2

construct and Rab5 or its S43N mutant. Single-molecule in-situ hybridization and IF for SNAP25 mRNA, an MBNL1 target, Rab5 and EGFP-MBNL1 WT or its C-term deletion mutant.

RESULTS: MBNL1 mRNA granules are located on early endosomes and the C-term of MBNL1 bi-directionally regulates its mobility with endosomes. Work in progress is to evaluate the role of the C-term of MBNL1 in endosome coupled local translation.

CONCLUSIONS: Our findings suggest that one of the mechanisms for MBNL1 to regulate mRNA cargo distribution and localized translation in neurons is endomembrane clustering.

FINANCIAL SUPPORT: NIH R01 NS114253 grant awarded to G.J.B and E.T.W.

P3.06. MULTICENTRIC STUDY OF LONGITUDINAL CHANGES IN NEUROMELANIN MRI SIGNAL AS A PROGRESSION MARKER IN PARKINSON'S DISEASE

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INTRODUCTION: Neuromelanin-sensitive MRI (NM-MRI) offers a promising avenue for non-invasive assessment of dopaminergic neuron integrity in Parkinson's disease (PD). Monitoring disease progression remains a major challenge in clinical practice and research.

AIM(S): This study aimed to evaluate NM-MRI markers as potential biomarkers of PD progression, with a particular focus on longitudinal changes in the substantia nigra (SN).

METHOD(S): A total of 483 participants from two cohorts were included: healthy volunteers, patients with isolated REM sleep behavior disorder (iRBD), and PD patients. All underwent 3T NM-MRI. Automated segmentation algorithms were used to extract SN volumes, signal-to-noise ratio (SNR), and contrast-to-noise ratio (CNR).

RESULTS: At baseline, PD patients showed significantly lower SN volume, SNR, and CNR compared to both controls and iRBD patients, indicating neuromelanin loss. Longitudinal analyses revealed progressive reductions in these markers across patient groups, with a notable effect of sex. These changes reflect the ongoing degeneration of dopaminergic neurons in PD.

CONCLUSIONS: NM-MRI provides a sensitive, non-invasive tool for tracking PD-related neurodegeneration. Its potential as a biomarker for disease progression and therapeutic response is reinforced by longitudinal evidence. Moreover, artificial intelligence proves essential for robust image analysis and may enhance future diagnostic precision. Further studies are warranted to validate these findings and optimize clinical applications.

FINANCIAL SUPPORT: The PhD is supported through dual funding from Novartis and the Michael J. Fox Foundation for Parkinson's Research.

P3.07. GUT–BRAIN AXIS MODULATION OF SYMPTOMS IN EARLY PARKINSON'S DISEASE: EVIDENCE FROM A PILOT STUDY

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INTRODUCTION: Growing evidence indicates that gastrointestinal (GI) dysfunction in Parkinson's disease (PD) can impact central nervous system function through the microbiota–gut–brain axis. However, the underlying mechanisms remain poorly understood.

AIM(S): To investigate whether GI symptoms in early PD are associated with changes in cortical activity, and whether this activity mediates the manifestation of PD symptoms.

METHOD(S): We examined 28 patients with early PD. GI symptoms were assessed using the Polish version of the Gastrointestinal Symptom Rating Scale (GSRS), clinical severity with the Unified Parkinson's Disease Rating Scale (UPDRS), and resting cortical bioelectrical activity using electroencephalography (EEG) with source analysis (eLORETA).

RESULTS: Source analysis revealed a significant negative correlation ($r=-0.42$, $p<0.05$) between GI symptom severity and alpha-band (8–12 Hz) activity, with the source localized in the bilateral occipital cortex (Brodmann Areas 17 and 18), as well as the posterior parietal cortex (BA 7). Mediation analysis of the relationship between GI symptoms (GSRS) and PD symptoms (UPDRS), with cortical activity (EEG in alpha bands) as the mediator, indicated distinct pathways: EEG activity significantly mediated the associations between GI symptoms and non-motor features such as speech disturbances ($p=0.021$) and daytime sleepiness ($p=0.019$). In contrast, motor symptoms like freezing of gait showed a direct relationship with GI severity ($p<0.001$), suggesting separate pathophysiological mechanisms.

CONCLUSIONS: Our findings suggest that GI dysfunction may influence resting-state cortical activity in early PD. This relationship may modulate distinct pathophysiological pathways, contributing to different symptom manifestations via the gut–brain axis.

FINANCIAL SUPPORT: Internal grant awarded by the University School of Physical Education in Wrocław in 2024, titled: "Does interval training on a cycle ergometer affect gut microbiota, gut-brain axis mechanisms, brain bioelectrical activity, and psychomotor functions in patients with Parkinson's disease?"

P3.09. IRON-CALCIUM CROSSTALK AND FERROPTOSIS IN LRRK2-LINKED PARKINSON'S DISEASE. NEUROPROTECTIVE ROLE OF MCU INHIBITION

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INTRODUCTION: Parkinson's Disease (PD) is a neurodegenerative disorder characterized by dopaminergic neuron (DN) loss in the substantia nigra. Dysregulated iron metabolism, mitochondrial calcium (Ca^{2+}) overload, and oxidative stress contribute to neurodegeneration. Ferroptosis, a form of regulated cell death dependent on iron and lipid peroxidation, has been implicated in PD pathogenesis.

AIM(S): To investigate ferroptosis in neuronal cells carrying pathogenic LRRK2 mutations and assess whether inhibition of the MCU alleviates ferroptosis-related damage.

METHOD(S): Ferroptosis was induced using RSL3 in LRRK2-mutant (G2019S) and control human neuronal cells. The cells were obtained from iPSc lines. Mitochondrial calcium was modulated using Ru360, a specific MCU inhibitor. Lipid peroxidation levels were measured using C11-BODIPY staining, and intracellular iron (Fe^{2+}) content was assessed using Assay Kit (Colorimetric). Expression of ferroptosis markers (GPX4, NRF2, HMGB1, HMOX1) was evaluated by qPCR and Western blot.

RESULTS: LRRK2-mutant neurons showed increased susceptibility to RSL3 compared to control cells, with elevated lipid peroxidation and iron accumulation. These cells also displayed downregulation of GPX4 and HMGB1 and upregulation of NRF2 and HMOX1. MCU inhibition with Ru360 restored redox balance, decreased lipid peroxidation, and improved neuronal survival, suggesting attenuation of ferroptosis.

CONCLUSIONS: Ferroptosis contributes to neurodegeneration in LRRK2-linked PD. MCU inhibition protects neurons likely by modulating ferroptosis pathways. Targeting MCU may represent a promising therapeutic approach for PD.

FINANCIAL SUPPORT: This study was supported by the National Science Centre (NCN), OPUS 25, project no. 2023/49/B/NZ4/02744 to JK).

P3.10. ESTIMATION OF THE THERAPEUTIC POTENTIAL OF THE ANTI-INFLAMMATORY DRUGS IN THE TREATMENT OF PARKINSON'S DISEASE

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INTRODUCTION: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra, with a key role of α-synuclein aggregation and neuroinflammation. Epidemiological observations and preclinical studies suggest that use of anti-inflammatory drugs (mainly non-steroidal anti-inflammatory drugs, NSAIDs) protect against PD. Unfortunately, the long term usage of NSAID causes numerous gastrointestinal side effects and is cardiotoxic. Preclinically data on NSAIDs in PD is limited to older, acute neurotoxic models, while rationale favors their use in prodromal PD stages – and conversely – in novel, progressive PD models like TIF1ADATCreERT2 genetic model, and the preformed α-synuclein fibril (PFF) injection model.

AIM(S): The study aimed to select a candidate among the available and developing anti-inflammatory drugs that could be investigated in such models.

METHOD(S): We searched the literature using the PubMed database for pharmacotherapeutic strategies for neuroinflammation in neurological diseases, focusing on their blood-brain barrier (BBB) permeability and the safety of their long-term use.

RESULTS: Publications have shown that the most popular strategies for treating neuroinflammation are still the inhibition of enzymes of various types of cyclooxygenases. Our attention was drawn to the compound MIP001, which showed therapeutic efficacy in animal models of inflammation and pain. In addition, the MIP001 showed no ulcerogenic effect and no other undesirable effects on the gastrointestinal tract. Despite the lack of studies on the penetration of BBB, MIP001 reduced locomotor activity, prolonged sleep time after hexobarbital, caused headaches and dizziness in pre- or clinical studies.

CONCLUSIONS: Because the higher safety after the long-term use of MIP001 and data suggesting its BBB penetration, MIP001 seems to be suitable candidate for further studies of its therapeutic potential in animal models of PD.

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).

P3.11. ENDOLYSOSOMAL DISRUPTION MODULATES ALPHA-SYNUCLEIN AGGREGATION IN A CELLULAR MODEL OF PARKINSON'S DISEASE

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INTRODUCTION: Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by motor symptoms such as rigidity and bradykinesia, which occur after the loss of approximately 50% of dopaminergic neurons in the SN/VTA region. This neuronal loss is a slow, progressive process that begins years before the onset of motor symptoms. Currently, no available treatment can halt this progression, and a lack of understanding of the mechanisms potentially responsible for neurodegeneration in PD hinders the development of effective therapies. The pathological aggregation and prion-like spreading of misfolded α-synuclein, and the formation of Lewy bodies, are considered key mechanisms. Therefore, elucidating the molecular mechanisms behind α-synuclein aggregation and its clearance may reveal promising therapeutic targets.

AIM(S): We aimed to examine the role of disruption of the endolysosomal pathway (ELP) in pathological α-synuclein aggregation and cell survival.

METHOD(S): We utilized an α-synuclein preformed fibrils (PFF) in vitro model in primary neuronal cultures. Disruption of the ELP was achieved by increasing endolysosomal pH using chloroquine (CQ; 5 μM, 10 μM, 12.5 μM). Additionally, we applied CRISPR-Cas9-mediated deletion of the lysosomal ion channel TMEM175, responsible for maintaining optimal lysosomal pH. Im-

munofluorescence staining for pS129-α-synuclein-positive aggregates and NeuN was used for analysis.

RESULTS: As expected, CQ at 10 μM and 12.5 μM decreased neuronal survival and impaired degradation of α-synuclein aggregates. Interestingly, 5 μM CQ showed potential neuroprotective effects. Surprisingly, TMEM175 ablation reduced the accumulation of pathological α-synuclein threefold.

CONCLUSIONS: The ELP appears to be an important factor in α-synuclein aggregation; however, the role of TMEM175 is complex and requires further investigation.

FINANCIAL SUPPORT: Funding: This research was funded by the National Science Centre, Poland (grant no. 2019/35/D/N27/03200, Sonata 15).

P3.12. NANOMATERIAL-BASED STRATEGIES AGAINST PARKINSON'S-RELATED A-SYNUCLEIN MISFOLDING AND AGGREGATION

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INTRODUCTION: α-Synucleinopathy is a well-recognized hallmark of several neurodegenerative disorders, including Parkinson's disease. Aggregates of α-synuclein spread into different neuronal populations during disease progression, which putatively contributes to a broad spectrum of symptoms such as sleep disturbances, motor dysfunction, depression, cognitive impairment, and others. In recent years, nanotechnology has emerged as a promising field for both the diagnosis and treatment of proteinopaties. In particular, Graphene Quantum Dots (GQDs) and Gold Nanoparticles (AuNPs) have shown potential in interfering with the fibrillization process of α-synuclein.

AIM(S): The primary aim of this study was to investigate the impact of these nanoparticles on α-synuclein aggregation using in vitro neuronal model of α-synucleinopathy.

METHOD(S): Graphene- or gold-based nanoparticles were administered to mice primary neuronal cultures (cortical, hippocampal, dopaminergic) on day 7, one hour after treatment with preformed fibrils (PFFs) of mouse recombinant α-synuclein. The cultures were then incubated for 7 days. After fixation with 4% PFA, the cells were stained using primary antibodies (NeuN, TH, and α-synuclein phospho-S129) and corresponding secondary antibodies (Alexa Fluor 488 and 647).

RESULTS: GQDs, applied at concentrations of 5 μg/mL and 10 μg/mL, effectively reduced the proportion of cells exhibiting pathological α-synuclein accumulation across all three types of neuronal cultures, without exerting any negative effects on neuronal survival. Similarly, AuNPs at 50 μg/mL demonstrated a comparable protective effect, which was observed in hippocampal and cortical neuronal cultures, also without compromising cell viability.

CONCLUSIONS: These findings indicate that GQDs and AuNPs can reduce pathological α-synuclein accumulation without affecting neuronal viability, supporting their potential as therapeutic agents in synucleinopathies.

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P3.13. INHIBITION OF ALPHA-SYNUCLEIN AGGREGATION AND ER STRESS RESCUES NEURODEGENERATION IN A 3D IN VITRO MODEL OF PARKINSON'S DISEASE

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INTRODUCTION: Parkinson's disease (PD) is the second most common neurodegenerative disorder. The major molecular event underlying the pathophysiology of PD is the abnormal accumulation of α-synuclein (α-syn) within the dopaminergic neurons of the midbrain, which results in the induction of endoplasmic reticulum (ER) stress conditions and PERK-mediated neural cell apoptosis.

AIM(S): The main objective of the present study was to determine the potential effect of the small-molecule inhibitors of α-syn aggregation and ER stress-mediated PERK signaling pathway against neurodegeneration in a novel, 3D in vitro model of PD.

METHOD(S): The effectiveness of the selected α-syn aggregation inhibitor (anle138b) and PERK inhibitor (AMG44) was assessed in a midbrain organoid model of

PD derived from a human iPSC line. Neurodegeneration was induced via incubation with the neurotoxin 6-hydroxydopamine (6-OHDA) and α-syn pre-formed fibrils (PFF). Cell viability was assessed by the CellTiter-Glo 3D assay, and the presence of α-syn aggregates was detected by immunofluorescence.

RESULTS: The cell viability analysis demonstrated a significant increase in ATP production in PD organoids treated with anle138b and AMG44, with the most prominent effect upon combining the two inhibitors. Immunofluorescence analysis revealed a significant decrease in phospho-α-syn and aggregated-α-syn levels after treatment with the compounds, and combining anle138b with AMG44 resulted in the greatest anti-aggregative effect.

CONCLUSIONS: Combination therapy with the selected α-syn and ER stress inhibitors effectively rescues neurodegeneration in the 3D in vitro model of PD. The results obtained may help develop the first disease-modifying therapy for PD.

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P3.14. A NOVEL ANTI-INFLAMMATORY COMPOUND REDUCE ASTROCYTIC ACTIVATION IN FIBRILLAR MODEL OF PARKINSON'S DISEASE

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INTRODUCTION: Epidemiological studies suggest that chronic use of NSAIDs may reduce the risk of developing Parkinson's disease (PD), likely through modulation of neuroinflammatory processes. However, their application for long-term treatment in PD would likely require sustained high-dose regimens, raising safety concerns. MIP001 is a novel anti-inflammatory compound with an excellent safety profile and therapeutic efficacy.

AIM(S): The aim of this preliminary study was to evaluate the potential of MIP001 to modulate neuroinflammation in PD-relevant in vitro models.

METHOD(S): Primary mouse astrocyte cultures were treated with fibrillar α-synuclein (PFF) and MIP001 at a concentration of 10 μM, followed by immunofluorescent staining, microscopic evaluation, and measurement of IL-1β, IL-6, and TNFα levels in the culture medium.

RESULTS: MIP001 reversed the morphological changes in astrocytes induced by PFF and pro-inflammatory cytokines (immunofluorescence analysis). It also significantly reduced the production of TNFα ($p<0.05$) by activated astrocytes, without affecting cell viability.

CONCLUSIONS: These preliminary data demonstrate the capability of MIP001 to modulate neuroinflammation in PD-relevant models, supporting further investigation into its neuroprotective potential in more complex systems, as well as detailed mechanistic studies.

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).

P3.15. INDUCING NEURODEGENERATION IN ZEBRAFISH TO PROBE FERROPTOTIC PATHWAYS IN PARKINSON'S DISEASE

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INTRODUCTION: To ensure adequate energy supply in the brain, iron levels are precisely regulated, as it is vital for the mitochondrial electron transport chain (ETC). In neurodegenerative diseases like Parkinson's, iron dysregulation can trigger ferroptosis, leading to neuronal loss and fueling disease progression. Using zebrafish (Danio rerio), an excellent in vivo model due to their optical transparency, genetic tractability, and conserved neurochemical architecture, we aim to establish strategies for inducing this pathological iron accumulation.

AIM(S): We aim to develop approaches to induce pathological iron accumulation and determine if ferroptotic stress directly mimics Parkinsonian-like dopaminergic neuronal death. To model Parkinson's and ferroptosis-like conditions using MPTP, RSL3, and transgenic PINK1 lines, while also developing a CRISPR-Cas9-based LRRK2 knock-in line with Ms. Sofia Baranykova.

METHOD(S): Our approach involves generating models of Parkinson's Disease and ferroptosis using PINK1 mutant transgenic lines, alongside creating and characterizing chemically induced models. We expose the 5dpf wild-type larvae to varying concentrations of MPTP, and the PINK1 models to mimic PD-like environment while RSL3/Erastin are aimed at disrupting iron homeostasis. To investigate the molecular mechanisms of ferroptosis, we will examine the expression of key markers like GPX4, HMOX1, HMGB1, and MCU. Furthermore, in our various models, we utilize Perl's Prussian Blue staining to identify iron buildup that correlates with ferroptotic progression.

RESULTS: Initial lab findings show that PINK1 mutants exhibit DA neuronal loss at 3 dpf, which is mitigated by inactivation of the mitochondrial calcium uniporter (MCU).

CONCLUSIONS: These coordinated strategies will lay the groundwork for establishing a zebrafish-based platform for studying neurodegeneration and ferroptosis ultimately facilitating future pharmacological screening. As the project is in its early stages, definitive conclusions are yet to be drawn.

FINANCIAL SUPPORT: This study is supported by the National Science Centre (NCN), OPUS 25, project no. 2023/49/B/NZ4/02744 to JK.

P3.16. THE INFLUENCE OF LIGHT POLLUTION ON SURVIVAL OF PD MODEL DROSOPHILA MELANOGASTER

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INTRODUCTION: Artificial light at night caused by streetlamps and man-made light is called light pollution. It's detrimental to our health, since organisms are not adapted to constant light exposure. Numerous studies have shown that cell death is enhanced after long term exposure to bright light, which is especially dangerous for patients with age-related diseases, like Parkinson's disease (PD). In PD patients retina undergoes neurodegeneration well before the onset of typical symptoms, resulting in their increased sensitivity to light pollution, which can contribute to worsening their symptoms. The Parkinson's disease is less prevalent in females, which indicates presence of differences in its development between the sexes. In this study we investigated the influence of different light conditions on survival of male and female PD model Drosophila melanogaster.

AIM(S): Determination of the sex differences in survival of PD model flies exposed to different light conditions.

METHOD(S): We performed survival assays on males and females of Drosophila melanogaster with park homozygous mutation under different light conditions: Light:Dark 12h:12h (LD); constant darkness (DD); Light:Dim Light 12h:12h (L-dim) and Light:Blue:Darkness 12:1:11 (LBD). Wild type flies Canton S and white (genetic background of the park mutant flies;) strains were tested in the same conditions as a control.

RESULTS: Flies kept in constant light conditions exhibited the shortest lifespan. However, we did not observe changes in wild type females under these conditions. Surprisingly, LBD conditions had pro-survival effect on wild type flies, while in park mutants it was age-related.

CONCLUSIONS: Female flies of all strains were less affected by light pollution than males and the constant exposure to light was the most detrimental for flies' survival. In addition, PD model flies were more sensitive to ALAN than controls.

P3.17. THE DOPAMINE IN GLUTAMATERGIC NEURONS AFFECTS SURVIVAL AND MOTOR ABILITIES OF DOROSPHILA MELANOGASTER

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INTRODUCTION: Parkinson's Disease (PD) is a neurogenerative illness that over 8 million patients in the world struggle with and only few percent of these cases have a known cause – meanwhile an effective therapy that stops the progress of symptoms does not exist. PD starts with degeneration of dopaminergic cells in the midbrain but in recent years it has been found that the mortality of these neurons drops significantly when they also display glutamatergic transmission. The neuroprotective properties of VgluT2 are theorized but the significance of dopamine (DA) in the overall role of these cells has still not been determined. In this study we examined the role of DA in glutamatergic cells of Drosophila melanogaster with the use of climbing and survival assays, as well as analysis of circadian and sleep cycles in males of vGlut>pleRNAi strain, which

has silenced the expression of tyrosine hydroxylase specifically in glutamatergic cells.

AIM(S): Determination of the significance of DA in the glutamatergic cells in Drosophila melanogaster brain.

METHOD(S): Flies kept in 12:12h light to dark conditions from vGlut>pleRNAi and control: vGlut-Gal4/+ and UAS-pleRNAi/+ strains underwent survival and climbing assays as well as monitoring of sleep rhythm and daily activity with the use of DAM system.

RESULTS: We have found that the survival of flies lacking DA expression in glutamatergic neurons was significantly decreased, compared with controls. The period of circadian locomotor activity was not affected, but the DA lacking flies showed longer and more frequent bouts of sleep throughout the day.

CONCLUSIONS: DA in glutamatergic neurons of Dorosphila melanogaster plays a role in the overall motor ability of the animal and its' overall fitness.

P3.19. ANTI-INFLAMMATORY EFFECT OF EGF-FGF EXOSOMES IN PTZ INDUCED SEIZURE MICE

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INTRODUCTION: Epilepsy is a common disorder of the central nervous system (CNS), which manifests as recurrent episodes of brain dysfunction caused by large-scale synchronous abnormal discharges of neurons. Neuroinflammation is a pathological stimulation which plays a critical role in epileptogenesis. Thus, targeted therapies aimed at neuroinflammation offer promising new approaches on epilepsy. Recent findings indicate that members of the FGF family influence epilepsy regulation. However, the protective effects of exsosomes containing enhanced EGF and FGF on neuroinflammation following seizure during epileptogenesis remain underexplored.

AIM(S): To investigate the protection effect of EGF-FGF enhanced exsosomes on after seizure neuroinflammation.

METHOD(S): In this study, we applied engineering enhanced EGF and FGF expression 293T-exosome (EGF-FGF.Exos) as treatment agent to PTZ induced seizures mice models.

RESULTS: In PTZ seizure mice model, EGF-FGF.Exos exhibited strong inhibitory effect on microglial activation and regulated microglial polarization towards the M2 phenotype while suppressed M1 polarization. RT-qPCR results demonstrated that EGF-FGF.Exos reduced the mRNA expression of central pro-inflammatory factors IL-1β, IL-6, and TNF-α in the animal model. HE staining showed that EGF-FGF.Exos pretreatment prevented neuronal loss in both the CA1 and CA3 regions of the hippocampus. Further experiment demonstrated that the inhibitory effects of EGF-FGF.Exos on pro-inflammatory factor expression, microglial activation, and M1 polarization pre-activation is caused by inhibiting the NF-κB pathway, and this inhibitory effect on after-seizure inflammation was reversed by PMA (NF-κB pathway agonist).

CONCLUSIONS: In conclusion, this current study demonstrated that EGF-FGF enhancing exosomes (EGF-FGF.Exos) inhibit inflammatory responses in PTZ seizure mice model by suppressing NF-κB pathway.

FINANCIAL SUPPORT: This study was supported by an industry collaboratory grant from Suzhou Zhiheng Biotechnology Co. Ltd.

P3.20. ZT-1A INHIBITION OF SEIZURE ACTIVITIES BY DUAL MODULATION OF BOTH NKCC1 AND KCC2 EXPRESSION IN EPILEPTIC MICE

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INTRODUCTION: Epilepsy is a chronic neurological disorder featuring recurrent and unprovoked seizures. GABA inhibition in adult CNS play an important role in epileptogenesis. GABA AR-mediated inhibition depends on the maintenance of the low intracellular [Cl-] concentration, which is mainly regulated by both Na+-K+-Cl- cotransporter-1 (NKCC1) and K+-Cl- cotransporter-2 (KCC2) in neurons. Previous studies have shown that both co-transporters are regulated by the WNK-SPAK-KCC2 signaling pathway and play important role in pathological states. ZT-1a is a selective SPAK inhibitor, supposed to negatively modulate NKCC1 and positively modulate KCC2 expression.

AIM(S): This study aims to explore whether ZT-1a can interrupt the epileptogenesis through inhibiting WNK-SPAK-NKCC1/KCC2 signaling pathway.

METHOD(S): We applied ZT-1a in either in vivo, pentylenetetrazol and pilocarpine models or in vitro cyclothiazide- induced cultured hippocampal neuron model. The changes of correlated signaling pathway proteins and GABAAR-mediated inhibition were investigated by a series of experiment such as Western Blots, electrophysiology recordings, immunostaining, etc. to provide theoretical basis for the potential therapeutic effect of ZT-1a.

RESULTS: We discovered that ZT-1a, (1) attenuated CTZ-induced epileptiform bursting activities in primary cultured hippocampal neurons, (2) decreased the seizure susceptibility to PTZ stimulation and alleviated pilocarpine-induced chronic spontaneous seizures by reversing seizure induced KCC2 down regulation as well as NKCC1 upregulation, and (3) the suppressive effect of ZT-1a on epilepsy attributed to the inhibiting of the WNK-SPAK phosphorylation during seizure activities.

CONCLUSIONS: Our current study demonstrated that ZT-1a inhibition of WNK-SPAK pathway capable of simultaneously preventing KCC2 deficit and NKCC1 enhancement during pathological stimulation related epileptogenesis. In conclusion, WNK-SPAK inhibitor ZT-1a may serve as a lead compound for future anti-seizure drug development.

P3.21. MUTATIONS AFFECTING KV2.1 SUBUNITS DISRUPT INHIBITORY NEUROTRANSMISSION

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INTRODUCTION: Voltage-gated potassium (Kv) channels are essential for maintaining the resting membrane potential. Mutations in Kv2.1 channels have been implicated in developmental epileptic encephalopathy. Previous analysis of mutations in zebrafish genes encoding the Kv2.1 subunits (kcnb1 and kcng4b) has shown their antagonising roles in the development of hollow organs.

AIM(S): In this study, we investigate the effects of kcnb1 mutations in zebrafish by examining the behaviour and neurophysiology of kcnb1 loss-of-function (LOF, kcnb1sq301) and gain-of-function (GOF, kcng4b-waw304) mutants.

METHOD(S): Zebrafish larvae at 5 days post fertilisation (dpf) were assessed using behavioural assays like spontaneous locomotor activity, and pentylenetetrazole (PTZ) exposure. Additionally, response to light was assessed via multiple behavioural paradigms. Quantitative PCR (qPCR) was performed for genes implicated in epilepsy and electrophysiological activity was recorded from the optic tectum and retina.

RESULTS: Both LOF and GOF mutants exhibit hypoactivity under light conditions. Upon exposure to 5 mM PTZ, locomotor activity increases and seizures start. Analysis using qPCR revealed increased c-fos and gad2 transcript levels, but decreased gabra1 transcript level, suggesting impaired inhibitory neurotransmission. Electrophysiology recordings from the tectum show spontaneous electrical activity in the mutants under baseline conditions. The LOF mutant also exhibited light sensitivity characterised by freezing responses under high-intensity light. Electroretinography revealed loss of retinal activity in the LOF variant.

CONCLUSIONS: These findings indicate that the defects in the Kv2.1 subunits predispose the larvae to PTZ susceptibility, affecting locomotor behaviour and leading to disruption of inhibitory neurotransmission. Moreover, a reduction in the photomotor response in the LOF variant, along with loss of retinal electrical activity, suggests photosensitivity and highlights the role of kcnb1 in visual processing.

FINANCIAL SUPPORT: This work has been supported by the Polish National Science Centre OPUS grant UMO-2020/39/B/NZ3/02729.

P3.22. ROLE OF CERT1 IN CONTROL OF MICROGLIA BIOLOGY IN MICE – RELEVANCE FOR COGNITIVE FUNCTIONS

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INTRODUCTION: Ceramides are lipids that serve as key structural components of cell membranes and regulate cell proliferation, differentiation, and apoptosis. Ceramide transfer proteins (CERTs) transport ceramide from the endoplasmic reticulum (ER) to the Golgi apparatus for sphingomyelin synthesis and signalling molecule production. CERTs are crucial for embryogenesis, brain development, and immunoglobulin-independent complement pathway activation. Recent studies show ceramides stabilize β- and γ-secretases, enzymes that

cleave amyloid precursor protein (APP) to generate toxic amyloid-β (Aβ). Post-mortem Alzheimer's disease brains exhibit increased ceramide and related enzymes, with decreased sphingomyelins in membrane lipid rafts. In vitro, CERT binds APP and modulates Aβ aggregation, while CERT-overexpressing mouse models show reduced Aβ formation and attenuated microglial proinflammatory responses.

AIM(S): To investigate CERT's cell-autonomous functions in microglia, given their role in Alzheimer's disease and lipid dysregulation.

METHOD(S): We generated mice with floxed CERT1 alleles and excised CERT1 in microglia using Cx3cr1-Cre. Memory performance, microglial morphology (high-resolution microscopy), and inflammatory markers were assessed.

RESULTS: Microglia lacking CERT1 adopted an inflammatory morphology with elevated iNOS and CD68 expression, correlating with cognitive deficits in declarative memory tasks.

CONCLUSIONS: RNAseq analysis of purified microglia will elucidate molecular mechanisms underlying this phenotype.

P3.23. NEUROPHYSIOLOGICAL MARKERS OF BRAIN INJURY IN ACUTE DISORDERS OF CONSCIOUSNESS

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INTRODUCTION: Disorders of consciousness (DOC) include coma, unresponsive wakefulness syndrome, minimally conscious state, and the recently added covert awareness. They result from acquired brain injuries such as trauma, anoxia, or stroke. Despite scientific advances, the neurophysiological mechanisms underlying DOC remain insufficiently understood, limiting diagnostic accuracy and prognostic capabilities.

AIM(S): This study aims to identify key neurophysiological and physiological patterns associated with DOC, taking into account etiological and other pathogenic factors.

METHOD(S): Retrospective data from ICU patients with acute DOC will be analyzed. This includes demographics, clinical scale scores, pupillary reflexes, CT scan descriptions, and blood markers (e.g., NSE, TSH, fT3, fT4, cortisol). EEG signals (resting-state and photic stimulation) will be analyzed for spectral properties and measures of neural criticality.

RESULTS: The study is in progress. Based on our previous results, we hypothesize that patients with more favorable diagnoses will show EEG spectral profiles with more pronounced theta and alpha oscillations, an antero-posterior gradient, and less pronounced 1/f activity in the spectrogram. We expect to identify clear differences in markers related to the dominant pathogenic mechanisms – namely anoxic or traumatic injury.

CONCLUSIONS: Identified neurophysiological and clinical biomarkers may contribute to a better understanding of the brain dynamics underlying disorders of consciousness, as well as support earlier and more accurate prognosis, enhancing care and outcomes in this vulnerable patient group.

FINANCIAL SUPPORT: This project was funded by the Priority Research Area FutureSoc and qLife under the program "Excellence Initiative – Research University" at the Jagiellonian University in Cracow.

P3.24. THE PROGRESSION OF ALS IN THE SKELETAL MUSCLES AFTER SWIM TRAINING IN FEMALE AND MALE MICE

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INTRODUCTION: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects the upper and lower motor neurons in the brain. It has been shown that the prevalence of ALS is greater in men, and they have shorter survival compared to women. ALS is incurable and treatments are focused on managing and reducing the symptoms of the disease to improve quality of life. The most effective physical activities are gentle exercises such as swimming to help strengthen the unaffected muscles. Even though the

protective effects of certain physical activity in animal models of ALS have been reported, these positive effects of training were only effective when training started before the appearance of the first symptoms of the disease in mice.

AIM(S): The aim of our study was to determine the protective effect of swim training applied after the onset of disease on skeletal muscle atrophy and the progression of ALS in female and male mice, and whether it will limit destructive changes in the skeletal muscles and prolong the life span.

METHOD(S): Mice (20 females, 20 males) were divided into four groups: before the onset of ALS, early stage of ALS, terminal untrained ALS, and terminal swimming-trained ALS (five times per week). Muscles were prepared for Transmission Electron Microscopy. Samples from the Tibialis Anterior (TA) and Quadriceps (Qua) muscles were dissected at 4°C at the end of the study and weighed to determine muscle atrophy.

RESULTS: The morphological changes such as sarcomere misaligned, enlarged mitochondria with vacuolization were observed in electrographs of TA and Qua muscles in both males and females. The obtained results showed that swim training prolonged the lifespan by approximately 12% and 6% in female and male ALS mice, respectively. In females, it was associated with maintaining 23% of body weight and showed a significant increase in the area (μm2) occupied by mitochondria in TA of the early onset group.

CONCLUSIONS: Swim training has positive effects on female ALS mice.

FINANCIAL SUPPORT: This study was supported by grants from the National Science Centre, Poland: 2020/39/B/NZ7/03366 and 2021/43/D/NZ7/00862.

P3.25. DOES MTOR-INDUCED BRG1 DEGRADATION INFLUENCE TSC-RELATED SYNAPTIC DYSFUNCTION?

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INTRODUCTION: Brg1 is an ATP-dependent catalytic subunit of the BAF chromatin remodeling complex. It impacts gene expression, stimulates DNA repair, facilitates RNA processing, and contributes to neuron development and synaptic activity. Through mass spectrometry analysis, we identified Brg1 as one of the nuclear interactors of the mTOR. mTOR plays a pivotal role in cellular metabolism and growth across diverse cell types. Dysregulation of mTOR has been implicated in mTORopathies, including TSC and epilepsy.

AIM(S): Our study aimed to investigate the nuclear mTOR–Brg1 interaction and its implications for neuronal development and disease.

METHOD(S): Primary neuronal culture, human-derived iPSCs, Western blots, immunofluorescence staining, calcium imaging, RNA-seq analysis

RESULTS: Using in vitro cultured rat neurons, our data confirmed an increased nuclear mTOR-Brg1 interaction following kainic acid (KA) treatment, highlighting mTOR-induced phosphorylation of Brg1. We observed that modulation of mTOR and the proteasome influenced the Brg1 nuclear presence, suggesting proteasome-mediated degradation of Brg1 in the nucleus upon KA treatment. Consistent with these findings, the downregulation of Brg1 expression was noted upon TSC2 loss, resulting in mTOR hyperactivation in neurons. Ca^{2+} imaging and network analysis revealed strong similarities between neurons lacking TSC2 and those deficient in Brg1. However, further investigation demonstrated that their synaptic parameters differed, and the RNA-seq analysis revealed involvement of different gene programs.

CONCLUSIONS: These observations suggest that although network activity is increased upon TSC2 and Brg1 loss, Brg1 and TSC2 largely regulate distinct transcriptional programs. Collectively, these findings provide new insights into the nuclear functions of mTOR in neurons, particularly in regulating Brg1 stability and activity.

FINANCIAL SUPPORT: The research was financed under the NCN MAESTRO grant 2020/38/A/NZ3/00447.

P3.26. INVESTIGATION OF THE ANALGESIC POTENCY, METABOLIC IMPACT, AND EFFECTS ON LOCOMOTOR ACTIVITY OF THE NOVEL HISTAMINE H_3 RECEPTOR ANTAGONIST, LINS01022, IN A MURINE MODEL OF NEUROPATHIC PAIN

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INTRODUCTION: Neuropathic pain is a chronic condition that is often resistant to standard analgesics. A growing body of evidence suggests that the histaminergic system is a potential therapeutic target for pain management. Here, we have used a novel compound LINS01022 (1-(2,3-dihydrobenzofuran-2-yl)methylpiperazines), characterised as a potent H3R antagonist (with nanomolar affinity pKi=8.2)).

AIM(S): To investigate the analgesic potency of novel H3R antagonist, LINS01022, and its influence on metabolic parameters and locomotor activity in a mouse model of neuropathic pain.

METHOD(S): Neuropathic pain was induced using the chronic constriction injury (CCI) to the sciatic nerve. At day 14th after nerve injury, mice received a single intraperitoneal (i.p.) injection of LINS01022 [10, 20, and 30 mg/kg]. The control group was injected with the vehicle. The presence of mechanical hypersensitivity was determined by a von Frey test, 15, 45, 90, and 150 min after injections. Metabolic parameters [consumption of oxygen; production of carbon dioxide; respiratory exchange ratio; expenditure energy, and locomotor activity (meters, speed)] were measured using the Promethion metabolic cages system. CCI-exposed mice were injected with LINS01022 [20 mg/kg] or vehicle and monitored for 72 hours in metabolic cages. Naïve animals served as healthy controls.

RESULTS: Our data revealed an analgesic effect of LINS01022 at all tested doses compared to vehicle-treated controls. The administration of LINS01022 did not show any disturbances in metabolic parameters and locomotor activity.

CONCLUSIONS: Our studies bring the first evidence for the analgesic potency of the novel H3R antagonist. Moreover, the new compound did not reveal any influence on metabolic parameters and locomotion. @e suggest that LINS01022 is a promising compound with analgesic potency and a favourable safety profile and can be used as a novel pharmacological tool to deepen our knowledge of the histaminergic system in pathological conditions of the central nervous system

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P3.27. A NEW MOLECULAR MECHANISM OF NEUROPLASTICITY

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INTRODUCTION: Neuronal activity-dependent transcription is essential for synaptic plasticity and cognitive function. Its disruption contributes to disorders such as autism, schizophrenia, and Alzheimer's disease, and can be exacerbated by environmental stress. RNA-binding proteins are increasingly recognized as key regulators of activity-dependent gene expression. Among them, YTHDC1, a nuclear reader of m6A-modified RNA, has emerged as a candidate, though its role in the brain remains poorly understood. Investigating YTHDC1 may uncover new mechanisms linking RNA processing to experience-driven transcription and learning.

AIM(S): This study aims to investigate the role of the RNA-binding protein YTHDC1 in regulating activity-dependent transcription and synaptic plasticity in the hippocampus.

METHOD(S): We used a conditional knockout approach to selectively delete Ythdc1 in the CA1 region of the adult mouse hippocampus. Cognitive function was assessed using behavioral paradigms including novel object recognition and contextual fear conditioning. To examine synaptic physiology, we performed ex vivo electrophysiology on acute hippocampal slices using whole-cell patch-clamp recordings. Network-level activity was evaluated through local field potential recordings, with a focus on gamma oscillations in the CA3 region.

RESULTS: YTHDC1-deficient mice exhibited impaired memory formation and retention, as indicated

by reduced object discrimination and freezing behavior. While intrinsic neuronal properties remained unchanged, local field potential recordings revealed decreased gamma oscillation power and peak frequency in the CA3 region, suggesting disrupted hippocampal network activity.

CONCLUSIONS: YTHDC1 is essential for hippocampal-dependent learning and synaptic function. These findings highlight a novel link between RNA processing and activity-dependent transcription, offering new insights into the molecular mechanisms underlying cognitive function and dysfunction.

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P3.28. PLASTICITY OF LAYER 1 INTERNEURONS OF THE MOUSE VISUAL CORTEX

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INTRODUCTION: The brain integrates internally generated information with external sensory inputs, and this is fundamental to adapting to dynamic environments. In primary sensory cortices, the most superficial Layer 1 (L1) plays a key role in integrating contextual (top-down) signals, which are modulated by local inhibitory GABAergic interneurons (INs). My research focuses on cannabinoid receptor type 1 (CB1), which we found to be expressed in neuron-derived neurotrophic factor (NDNF)-positive L1 INs of the mouse primary visual cortex (V1). CB1 modulates synaptic transmission through retrograde endocannabinoid (eCB) signaling, potentially serving as a mechanism to gate top-down signals to pyramidal neurons (PNs) during visual processing.

AIM(S): We hypothesize that CB1-mediated plasticity of dendritic inhibition from L1 INs gates contextual signals to PNs during sensory input. We aim to reveal the role of CB1-mediated plasticity of a specific V1 circuit in visual perception.

METHOD(S): Fluorescent in situ hybridization (FISH), Immuno-histochemistry (IHC), patch-clamp electrophysiology in acute brain slices, optogenetics, in vivo 2-photon (2p) Ca imaging.

RESULTS: Using FISH, we found that ~70% of NDNF L1 INs express CB1. We confirmed CB1-mediated plasticity at L1 IN-L2/3 PN synapses using optogenetics and multiple patch-clamp electrophysiological recordings in acute cortical slices. Preliminary results from 2p-Ca imaging of L1 NDNF INs of head-fixed mice suggest that NDNF INs are more strongly recruited by non-visual spontaneous activity upon genetic knock-down of CB1 in L1 NDNF INs. We are presently analyzing the role of CB1 in L1 INs during the processing of visual stimulations.

CONCLUSIONS: Our findings indicate an unexpected source of activity-dependent GABAergic plasticity modulating slow feed-forward inhibition triggered by top-down, contextual signals in V1. This mechanism can play a significant role in visual perception.

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P3.29. NUCLEUS INCERTUS CONNECTIVITY WITH MIDBRAIN CENTRES – IMPLICATIONS FOR STRESS AND REWARD PROCESSING

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INTRODUCTION: The brainstem nucleus incertus (NI) plays a role in processing aversive stimuli and regulating the stress response. Our previous research has shown that this structure also modulates the activity of midbrain dopaminergic neurons in rats by providing inhibitory input to the ventral tegmental area (VTA). In addition to the VTA, the NI's projections also extend to the rostromedial tegmental nucleus (RMTg), which serves as the primary inhibitory input to the dopaminergic system in the midbrain.

AIM(S): The aim of this study was to better understand the circuit's anatomy, physiology and function, using advanced neuroscientific research techniques.

METHOD(S): To gain an understanding of the circuit's anatomy, two retrogradely transported viral vectors, each carrying a gene for a different fluorescent protein, were injected unilaterally into the VTA and RMTg of Sprague-Dawley rats. Next, electrophysiological recordings in urethane-anaesthetized rats were conducted after the administration of two viral vectors: one retrograde vector, containing the Cre recombinase gene, was injected into the VTA or RMTg, and another vector, carrying Cre-dependent genes for a light-sensitive opsin, was targeted to the NI. Lastly, preceding behavioural experiments, retrograde viral vectors carrying YFP were injected bilaterally into the animals' RMTg. Following a stress-induction procedure in operant conditioning chamber, anti-cFos immunostaining was performed.

RESULTS: The results indicate that, in contrast to the VTA, the RMTg receives bilateral input from the NI. Electrophysiological findings further demonstrate functional effects of this NI-derived innervation on both midbrain structures. Additionally, the behavioural data shed light on a potential overlap between the c-fos-reactive NI subpopulation and the neurons projecting to the RMTg.

CONCLUSIONS: Taken together, these findings suggest that NI-derived innervation of midbrain structures constitutes a complex network that plays a role in regulating stress and reward processing.

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P3.30. RELAXIN-3 AND OXYTOCIN SIGNALING IN THE VENTRAL DENTATE GYRUS: DIVERGENT NEUROMODULATORY ROLES IN STRESS- AND ANXIETY-RELATED CIRCUITS

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INTRODUCTION: Oxytocin (OXT) and relaxin-3 (RLN3) are neuropeptides that exert largely opposing effects on neuronal circuits controlling social, stress, and anxiety-related behaviors. OXT enhances social bonding and attenuates stress, whereas RLN3 promotes anxiety and social avoidance. However, how these signaling systems interact, particularly within the ventral dentate gyrus (vDG) of the hippocampus, an area highly involved in the control of social memory and interactions, remains poorly understood.

AIM(S): We aimed to elucidate the functional interaction between OXT and RLN3 and their receptors (OXTR and RXFP3, respectively) systems.

METHOD(S): We combined anatomical, molecular, and electrophysiological approaches in rats, alongside complementary anatomical studies in the human hippocampus.

RESULTS: Multiplex in situ hybridization in both rat and human DG revealed moderate co-expression of OXTR and RXFP3 mRNAs within vGAT1-positive neurons. Interestingly, immunohistochemistry staining results showed dense RLN3-containing fibers, but a lack of OXT-expressing fibers innervating rat vDG. Multielectrode array recordings showed that OXT caused an increase in neuronal firing, whereas RLN3 suppressed it; when applied together, each peptide's effect was significantly attenuated. Pharmacological experiments using selective OXT and RLN3 receptors agonists (TGOT for OXTR, A2 for RXFP3) demonstrated that blockade of KCNQ channels with XE991 reduced both OXTR- and RXFP3-mediated responses. Consistent with this, HiPlex in situ hybridization confirmed co-expression of RXFP3, OXTR, and KCNQ2 mRNAs in individual rat vDG neurons.

CONCLUSIONS: Together, these results identify the vDG as a critical hub where OXT and RLN3 exert antagonistic effects via KCNQ channels to shape neuronal excitability and, ultimately, stress, anxiety, and social behaviors. The conservation of OXTR and RXFP3 co-expression patterns in human DG underscores the translational relevance of these findings.

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P3.31. DIVERGENT EFFECTS OF OXYTOCIN AND RELAXIN-3 SIGNALING IN THE VENTRAL DENTATE GYRUS: A NEURONAL BASIS FOR ANXIETY DISORDERS

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INTRODUCTION: Anxiety disorders are among the most prevalent psychiatric conditions, with the ventral dentate gyrus (vDG) of the hippocampus playing a key role in their pathophysiology. Oxytocin (OXT) and relaxin-3 (RLN3) are crucial neuropeptides that regulate neuronal circuits involved in contextual processing, social interaction, stress, and anxiety-related behaviors. Activation of the OXT/oxytocin receptor (OXTR) system produces anxiolytic effects, while activation of the RLN3/relaxin-3 receptor (RXFP3) system has opposing, anxiogenic actions.

AIM(S): Despite their distinct roles, the interplay between the OXT and RLN3 signaling in key anxiety-related brain regions such as the vDG remains underexplored. This study aimed to examine the influence of OXT and RLN3 on the neuronal activity in the rat vDG, aiming to uncover mechanisms underlying anxiety disorders.

METHOD(S): Viral-based neural tract tracing was used to identify RLN3-positive fibers originating from the nucelus incertus (NI) in the vDG. HiPlex in situ hybridization (ISH) was applied to detect RXFP3 mRNA-expressing cells and assess co-expression with inhibitory neuron markers, including vesicular GABA transporter (vGAT1), somatostatin, and OXTR mRNA. Additionally, whole-cell patch-clamp ex vivo recordings were conducted to examine the effect of OXT alone and in the presence of RLN3 on vDG granule cell activity.

RESULTS: RLN3-positive fibers from the NI innervated both the hilus and inner molecular layer of the vDG in a similar pattern. HiPlex ISH showed RXFP3 mRNA co-expression with vGAT1 and somatostatin, and moderately with OXTR mRNA. Electrophysiological recordings revealed that these neuropeptides altered vDG granule cell activity.

CONCLUSIONS: In summary, our findings suggest that OXT and RLN3 exert opposing effects on neuronal activity in the vDG, which may represent a neuronal substrate for the modulation of stress, anxiety, and social behavior. Moreover, these results highlight the possible role of their interaction in anxiety-related disorders.

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P3.32. SOCIAL BRAIN: THE ROLE OF RELAXIN-3 AND OXYTOCIN SYSTEMS IN REGULATING SOCIOSEXUAL BEHAVIOUR

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INTRODUCTION: The brainstem nucleus incertus (NI) plays a key role in stress, anxiety, and social interactions. It is the main source of the neuropeptide relaxin-3 (RLN3), which activates its receptor RXFP3. The NI sends dense RLN3ergic projections to the forebrain bed nucleus of the stria terminalis (BNST), a region critical for regulating anxiety, stress, and social and sexual behaviours. The BNST also shows high expression of oxytocin (OXT) receptor OXTR and RXFP3. However, the molecular profiles of the BNST and NI, especially regarding the RLN3/RXFP3 and OXT/OXTR systems and their roles in sociosexual behaviour, remain largely unknown.

AIM(S): The study aimed to elucidate the role of NI in sociosexual behaviour in male and female Sprague-Dawley rats. It sought to examine the distribution of RLN3-positive fibres in BNST and the sensitivity of BNST neurons to RLN3 administration.

METHOD(S): A sociosexual behavioural test was conducted, followed by c-Fos immunohistochemical staining in the NI. Viral-based anterograde tract tracing was used to identify the source of RLN3-ergic fibres in the BNST. Patch-clamp ex vivo recordings with RLN3 administration were performed on BNST neurons to assess their sensitivity to the peptide.

RESULTS: A greater density of c-Fos-positive cells was found in the NI of males that interacted with females, compared to control males, while no such difference was observed between experimental and control groups in females. RLN3-containing fibres from the NI were colocalized in the BNST. RLN3 administration during patch-clamp recordings induced a whole-cell outward current in BNST neurons.

CONCLUSIONS: These observations suggest the possible involvement of the NI in sociosexual behaviours control in males and indicate that the BNST remain under the influence of RLN3/RXFP3 and OXT/OXTR systems. The patch-clamp experiment provides evidence that the BNST is sensitive to RLN3 and that RLN3 has a pronounced inhibitory influence on its neuronal activity.

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P3.33. ELECTROPHYSIOLOGICAL AND BEHAVIORAL INSIGHTS INTO THE IPN-VHPC CIRCUIT UNDERLYING SOCIAL STRESS RESPONSES

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INTRODUCTION: The interpeduncular nucleus (IPN) is a key regulator of anxiety and social behavior, characterized by a dense expression of the TrkA receptors, which bind nerve growth factor (NGF). Social stress enhances brain NGF levels, modulating IPN activity. The IPN forms functional connection with the ventral hippocampus (vHPC), a hub for social and anxiety-related signalling and a source of NGF.

AIM(S): Despite emerging evidence of the IPN–vHPC interaction, the specific behavioral and mechanistic contributions of this pathway remain unclear. Therefore this study aimed to characterise IPN neurons innervating the vHPC at electrophysiological and functional levels.

METHOD(S): Electrophysiological properties and NGF sensitivity of IPN neurons were examined using whole-cell patch clamp and multielectrode array recordings (MEA). Viral based tract-tracing was employed to characterize IPN-vHPC innervation. Resident intruder test was used to determine if IPN neurons innervating vHPC encode aggression-related information.

RESULTS: Whole-cell patch clamp recordings from slices containing the anterior IPN revealed no detectable response to NGF application. Tract-tracing studies identified numerous cells innervating the vHPC, particularly within the rostral (IPR) and lateral (IPL) subnuclei of the IPN. To further characterize this population, a series of experiments incorporating optogenetic tagging were conducted, revealing a subset of neurons responding with inward whole-cell currents to NGF administration. Complementary MEA recordings demonstrated both inhibition and excitation of IPN neurons after NGF application. The resident-intruder test revealed increased c-Fos expression in the group exposed

to resident's smell and after social defeat, identifying the activation of a distinct neuronal population in IPR.

CONCLUSIONS: Our findings unveil direct innervation of the vHPC by IPN subnuclei, along with NGF-mediated modulation of a subset of IPN neurons. Notably, the IPR appears to be selectively activated by social stress.

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P3.34. EXPLORING THE NEUROCHEMICAL PROFILE AND FUNCTIONAL CONNECTIVITY OF THE NUCLEUS INCERTUS–VENTRAL HIPPOCAMPUS CIRCUIT: POTENTIAL ROLE IN ANXIETY REGULATION IN RATS AND HUMANS

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INTRODUCTION: The ventral hippocampus (vHPC) plays a pivotal role in regulating stress and anxiety responses, with disruptions within its interneuron network strongly linked to clinical anxiety-related disorders. One major input to the vHPC originates from the pontine tegmental nucleus incertus (NI), which is the main source of the neuropeptide relaxin-3 (RLN3) in the rat brain. Notably, sustained activation of the RLN3 receptor, RXFP3, in the vHPC has been shown to increase anxiety and social avoidance, though the underlying neurobiological mechanisms remain unclear.

AIM(S): Therefore, this study aimed to investigate the neurochemical profile and functional connectivity of the NI-vHPC pathway in the rat brain, alongside anatomical investigations in the human hippocampus.

METHOD(S): RLN3 fiber distribution and their origin in the ventral dentate gyrus (vDG) were investigated using immunohistochemistry and viral-based neural tract-tracing. RXFP3 mRNA-expressing neurons were characterized using HiPlex in situ hybridization (ISH) and their sensitivity to RLN3 was examined using ex vivo multi-electrode array recordings.

RESULTS: Immunohistochemistry revealed abundant RLN3-positive fiber innervation in the vDG of the rat hippocampus. Viral-based neural tract-tracing further confirmed a predominantly ipsilateral innervation of the rat vDG by RLN3 NI neurons. ISH studies showed that RXFP3 mRNA-expressing neurons in the rat vDG co-express vesicular GABA transporter (vGAT) and somatostatin mRNA. Importantly, ISH with human anterior hippocampal sections also revealed co-expression of RXFP3 and somatostatin mRNA. Finally, electrophysiological recordings ex vivo using multi-electrode arrays demonstrated an inhibitory effect of RXFP3 activation on the rat vDG network activity.

CONCLUSIONS: Collectively, these findings indicate a direct influence of RLN3 on GABAergic vDG interneurons in rat and human, which may underlie the effects of RLN3/RXFP3 signalling on anxiety-related behaviours.

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P3.35. ANGIOMOTIN-LIKE 1 – A NEW PLAYER IN THE BRAIN PHYSIOLOGY

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INTRODUCTION: Angiomotin-like 1 (AMOTL1) belongs to the angiomotin family of proteins which functions have been primarily characterized in vertebrate epithelial cells: the proteins regulate adhesive contacts, cell migration, polarization, and activity of the HIPPO pathway. However, the function of AMOTL1 in the central nervous system remains widely unknown. To characterize the role of AMOTL1 in the brain we generated a systemic knock-out mice (AMOTL1 KO) in which we observed a locomotor hyperactivity, an increased turning behavior, a decreased anxiety, and sensitivity to a low dose of amphetamine – most of the phenotypes can be linked with mouse models of different neuropsychiatric disorders.

AIM(S): The aim of the study was to characterize the functions of AMOTL1 in the brain.

METHOD(S): We performed a set of experiments using primary neurons cultures. It allowed to describe the morphological features of the neurons. Additionally, using tissue samples, we quantified the neurotransmitters of interest level via immunoenzymatic approach, as well as we immunodetected by Western blot the amount of specific proteins. The genes of interest expression has been quantified by RT-qPR.

RESULTS: Our experiments demonstrate that AMOTL1 plays important functions in the central nervous system. We identified important centers in the brain where AMOTL1 appears to have critical functions and discovered that lack of AMOTL1 leads to altered synaptic organization and neurotransmitters alterations. The changes were associated with behavioral impairments of mutant mice observed in various behavioral tests. Collectively, our studies highlight AMOTL1 as a potential key player in regulating pathological processes observed in various neuropsychiatric disorders.

CONCLUSIONS: The study clearly demonstrates the importance of AMOTL1 in proper functioning of the brain. We characterized behavioral abnormalities in AMOTL1 mutant mice and provided results revealing potential molecular and cellular causes.

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P3.36. DIFFERENCES IN TONIC CURRENT DENSITY DUE TO PLASTICITY INDUCTION IN SST+ CELL SUBTYPES

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INTRODUCTION: There are two main types of neuronal inhibition: phasic and tonic one. Recent findings show that N-methyl-D-aspartic acid (NMDA) treatment induces plastic changes in tonic inhibition in hippocampal interneurons including somatostatin-expressing (SST+) cells which, through interactions with other neurons, play a crucial role in regulating the hippocampal neuronal network (Wyroślak et al., 2023).

AIM(S): Herein, we investigated the influence of heterosynaptic inhibitory long-term potentiation (iLTP) induction via transient NMDA applications on tonic current density in subtypes of SST+ cells.

METHOD(S): Experiments were conducted on brain slices (hippocampal CA1 region) from SST-Cre x Ai14 mice. Tonic currents were measured using patch-clamp and pharmacology. Cells were filled with biocytin and imaged using confocal microscopy.

RESULTS: Our data allowed us to divide SST+ cells into two subgroups: oriens-lacunosum/moleculare (OLM) and non-OLM cells. OLM cells showed a higher tonic current density (0.78 ± 0.40 pA/pF, $p<0.05$) in the control group than non-OLM cells (0.21 ± 0.02 pA/pF, $p<0.05$). Induction of plasticity with NMDA caused an increase in tonic current density in OLM cells (0.92 ± 0.48 pA/pF, $p > 0.05$) but in non-OLM cells we observed a decrease in tonic inhibition (0.13 ± 0.01 pA/pF, $p<0.05$).

CONCLUSIONS: We conclude that distinct subgroups of SST+ cells show differences in tonic inhibition and NMDA treatment causes the opposite plastic effect on tonic currents in OLM cells and other SST+ cells. These differences indicate functional heterogeneity of SST+ neurons and their distinct roles in local network regulation.

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P3.37. NONASSOCIATIVE ILTD AT INTERNEURON–INTERNEURON SYNAPSES IN THE HIPPOCAMPUS

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INTRODUCTION: Plasticity in the brain comprises a range of mechanisms that alter synaptic strength and composition and plays a key role in memory encoding. Plastic changes may arise from both: coordinated activity of presynaptic stimulation and postsynaptic responces, and modifications not involving presynaptic activity. Also, plasticity at excitatory and inhibitory inputs onto principal cells has been extensively investigated, while long-term plastic changes at interneuron–interneuron (I-I) synapses remain less well characterized.

AIM(S): We investigated plastic changes at I-I synapses onto interneurons located in the stratum oriens of the hippocampus.

METHOD(S): Optogenetics was used to selectively activate VIP-positive presynaptic interneurons and applied protocols: with and without presynaptic stimulation (spike timing-dependent plasticity and depo-

larization-only protocols). Postsynaptic INs were characterized by electrophysiological properties and morphological features.

RESULTS: Our data showed uniform responses across all tested timing intervals (+60 ms, 0 ms, -60 ms) in STDP protocols. Postsynaptic fast-spiking interneurons exhibited no significant plastic changes, while OLM and other non-FS interneurons developed long-term depression of inhibitory inputs (iLTD). The lack of timing-specific changes suggests a nonassociative form of plasticity that does not require VIP-IN activation. Experiments omitting presynaptic stimulation further supported this conclusion. We then examined two molecular pathways potentially involved in this plasticity. Blocking endocannabinoid signaling with a CB1 receptor antagonist did not prevent iLTD induction. In contrast, blocking L-type voltage-gated Ca^{2+} channels alone did not abolish iLTD, but co-application of L- and T-type channel blockers significantly reduced the magnitude of I-I iLTD.

CONCLUSIONS: Hereon we demonstrate that inhibitory synapses onto interneurons in the stratum oriens undergo nonassociative forms of synaptic plasticity, likely mediated by postsynaptic Ca^{2+} influx.

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P3.38. BIDIRECTIONAL MODULATION OF GABAERGIC SYNAPTIC TRANSMISSION AND PLASTICITY IN VIP INTERNEURONS BY D2-FAMILY DOPAMINE RECEPTORS

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INTRODUCTION: Dopaminergic neuromodulation is a crucial factor influencing cognitive functions and information processing in the hippocampus. Its role has been demonstrated in shaping the synaptic plasticity of excitatory synapses as well as in memory and learning processes (Tsetsenis et al., 2023). However, it is unknown if dopaminergic receptors could also modulate the inhibitory synaptic transmission and its long-term potentiation (iLTP) in different classes of interneurons (INs).

AIM(S): Herein, we examine the role of D2-family dopamine receptors (D2Rs) in modulating GABAergic synaptic transmission and its plasticity in two distinct groups of vasoactive intestinal peptide-expressing (VIP) INs.

METHOD(S): We performed patch-clamp recordings of miniature inhibitory postsynaptic currents (mIPSCs) in hippocampal brain slices from VIP-tdTomato mice, followed by anatomical analyses of VIP INs. To check the role of D2Rs in inhibitory transmission, we used D2R agonist (quinpirole) or antagonist (sulpiride). We also evoked iLTP by NMDA treatment in control conditions and after blocking or enhancing D2Rs activity.

RESULTS: We found that, in the presence of quinpirole, mIPSCs amplitude was significantly potentiated in type 3 interneuron-specific (IS3) VIP INs (118±4% of control) but not in basket cells (BC) VIP INs (94±7%). In contrast, application of sulpiride led to the opposite effects (IS3: 104±4%; BC: 116±5%). The amplitude increase was also coupled with a prolongation of mIPSCs duration (IS3+quinpirole: 129±9%; BC+sulpiryde: 118±7%). Furthermore, we discovered that exposure to NMDA induced iLTP in IS3 VIP INs (120±5%) and iLTD in BC VIP INs (78±3%). Moreover, in IS3 VIP INs iLTP was accompanied by prolonged mIPSCs decay (125±7%). Finally, this form of inhibitory plasticity was occluded by bath application of quinpirole (95±4%) indicating a key role of D2Rs.

CONCLUSIONS: Altogether, our results show that D2Rs signaling interferes with GABAergic transmission and plasticity in the hippocampus in cell-type specific manner.

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P3.39. ADRENERGIC MODULATION OF INTRINSIC CORTICAL EXCITABILITY IN PREFRONTAL PYRAMIDAL NEURONS

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INTRODUCTION: Rebound depolarization (RD) is a neuronal mechanism in which membrane depolarization occurs following a period of hyperpolarization. This phenomenon often results in a burst of action potentials and effectively transforms inhibitory input into excitatory output, thereby influencing downstream synaptic activity. Despite its functional significance, the mechanisms underlying RD in cortical neurons remain incompletely understood.

AIM(S): The present study aimed to investigate the role of adrenergic receptor activation—specifically α1-, α2-, and β-adrenoceptors—in the generation of RD in pyramidal neurons of the medial prefrontal cortex (mPFC).

METHOD(S): Experiments were conducted on layer V pyramidal neurons in acute brain slices from adult male rats (58–65 days old). Whole-cell current-clamp recordings were performed in the presence of tetrodotoxin (TTX), DNQX, DL-AP5, and picrotoxin to block voltage-gated sodium channels and synaptic glutamatergic and GABAergic transmission, ensuring complete synaptic isolation. The effects of noradrenaline, cirazoline (α1-agonist), clonidine (α2-agonist), and isoproterenol (β-agonist) were assessed.

RESULTS: Application of noradrenaline, cirazoline, and isoproterenol reliably induced RD in mPFC pyramidal neurons. In contrast, application of clonidine did not produce significant changes in membrane potential or elicit RD.

CONCLUSIONS: These findings suggest that activation of α1- and β-adrenoceptors promotes RD in layer V pyramidal neurons of the mPFC, whereas α2-adrenoceptor activation does not. This receptor-specific modulation highlights a potential mechanism through which adrenergic signaling can influence cortical excitability and information processing.

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P3.40. ONCOMETABOLITE D-2HG ALTERS M6A RNA MODIFICATIONS IN IDH MUTANT GLIOMAS TO PROMOTE NEUROGLIOMAL SYNAPTIC SIGNALING

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INTRODUCTION: Isocitrate dehydrogenase (IDH) mutations define a molecular subset of diffuse gliomas that exhibit slower growth yet ultimately progress and resist treatment. These mutations confer a neomorphic enzymatic activity that leads to accumulation of the oncometabolite D-2-hydroxyglutarate (D-2HG), which reshapes the epigenetic landscape by inhibiting α-ketoglutarate–dependent dioxygenases. While the effects of D-2HG on DNA and histone methylation are well documented, its influence on RNA demethylases regulating N6-methyladenosine (m6A) remains largely unexplored.

AIM(S): Investigating how intracellular D-2HG alters the m6A epitranscriptome of glioma cells and facilitates tumor-neuron communication in IDH-mutant gliomas.

METHOD(S): Using IDH-mutant glioma cells treated or not with an IDH inhibitor to deplete D-2HG, we perform direct RNA nanopore sequencing to map transcriptome-wide m6A changes.

RESULTS: Our preliminary data suggest that D-2HG inhibits FTO activity and promotes m6A accumulation, particularly on transcripts related to synaptic signaling, leading to their stabilization. The functional consequences of these modifications will be assessed through co-culture of neurons with IDH-mutant glioma cells, combined with electrophysiology and calcium imaging. We aim to demonstrate that glioma m6A remodeling enhances glutamatergic signaling toward tumor cells, thereby promoting their proliferation and invasion. Silencing of candidate m6A-modified transcripts will be used to validate their contribution to these effects.

CONCLUSIONS: By unveiling a novel mechanism through which a tumor-derived metabolite alters RNA regulation to support glioma progression, this study highlights m6A as a key player in neurogliomal crosstalk and a potential therapeutic axis in IDH-mutant gliomas.

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P3.41. KAINATE-MEDIATED SIGNALLING IN THE OLFACTORY BULB IS INVOLVED IN THE GENERATION OF KETAMINE-ENHANCED HIGH-FREQUENCY OSCILLATIONS (130-180 HZ) IN FREELY MOVING RATS

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INTRODUCTION: N-methyl-D-aspartate receptor (NMDAR) antagonists such as ketamine are known to induce abnormal high frequency oscillations (HFO; 130-180 Hz) across different rat brain areas. However, the neural mechanisms behind the generation of this rhythm remain poorly understood.

AIM(S): This study aimed to determine the role of the olfactory bulb (OB) in the generation of ketamine-enhanced HFO and whether non-NMDA glutamate receptors, specifically AMPA or kainic acid receptors, contribute to the generation of HFO.

METHOD(S): Adult male Wistar rats were chronically implanted with electrodes and guides in the OB and electrodes in the ventral striatum (VS), prefrontal cortex (PFC), parietal cortex (ECoG-P), and frontal cortex (ECoG-F). Local field potentials were analyzed before and after intraperitoneal injection of ketamine (25 mg/kg). To test the involvement of AMPA/kainate receptors, CNQX/NBQX/UBP310/IEM1925 dihydrobromide (0.5 µg) was infused directly into the OB followed by the systemic administration of ketamine.

RESULTS: Systemic ketamine increased the power of HFO in the OB more strongly than VS or cortical areas (N=19). In a second study (N=7) we found that local OB infusion of CNQX or NBQX significantly reduced ketamine-enhanced HFO power locally and in the VS and PFC. In a third group (N=6) we found OB infusion of a KA antagonist (UBP310) reduced the power of ketamine-enhanced HFO power locally and in VS to a greater extent that the AMPA antagonist (IEM1925).

CONCLUSIONS: Ketamine-enhanced HFO recorded in the VS and PFC is dependent on HFO generated in the OB. Further, within the OB we show that the generation of HFO depends primarily on stimulation of kainate receptors.

FINANCIAL SUPPORT: The authors wish to thank NCN (grant 2021/41/B/NZ4/03882) for funding this project.

P3.42. BRAIN TSUNAMIS – MODELING MIGRAINE-LINKED SPREADING DEPOLARIZATION AND THE EFFECT OF VALPROATE

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INTRODUCTION: Migraine is a common chronic neurological disorder, affecting nearly 15% of the global population. It is marked by recurrent headaches, often arising with migraine aura (visual disturbances, sensory changes and speech or language difficulties) before the headache. Cortical spreading depression or depolarization (CSD) is considered to be an underlying mechanism of the migraine aura. It presents as waves of abrupt and sustained mass depolarization in neurons that propagates across the cortex.

AIM(S): This project aimed to induce CSD in acute mouse brain slices as a model to study the pathology underlying migraine aura. Moreover, the effect of valproate, used clinically as a preventive anti-migraine drug, was studied on the course of CSD, in order to examine the mechanism of action of the drug.

METHOD(S): High-concentration KCl puffs of increasing duration were applied to cortical layer 2/3 in acute mouse brain slices to induce CSD. Simultaneously, the membrane potential of a nearby pyramidal neuron was recorded (whole-cell current-clamp) as well as an intrinsic optical signal. CSD was observed as a spreading wave in the optical signal and a long-lasting depolarization in neurons. Recordings in valproate and control conditions were compared to access differences in CSD course and the mechanism of action of the drug.

RESULTS: CSD can be induced ex vivo in acute mouse brain slices and used as a therapeutic target for migraine prophylactic drugs. Valproate did not inhibit CSD initiation or the duration of the depolarization at half amplitude. Although it decreased the peak amplitude of depolarization of neurons during CSD, the acute treatment was ineffective to prevent CSD.

CONCLUSIONS: Pharmacological modulation of CSD could help in diminishing neurological conditions such as migraine with aura. Mechanisms of CSD initiation and treatment options can be studied ex vivo in acute mouse brain slices. Although valproate acute treatment did not prevent CSD, it may be effective in migraine prophylaxis during chronic use.

FINANCIAL SUPPORT: ERC starting grant 802354 “NovelNMDA”.

P3.43. ELECTROPHYSIOLOGICAL PROFILING OF SYNAPTIC DYSFUNCTION IN TOXIN-INDUCED AND GENETIC ZEBRAFISH MODELS OF PARKINSON'S DISEASE

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INTRODUCTION: Parkinson's disease (PD) is characterized by late-onset motor symptoms resulting from the loss of dopaminergic neurons (DNs). Increasing evidence suggests that early synaptic and axonal dysfunction—driven by mitochondrial calcium dysregulation and ferroptosis—precedes neuronal death. Previous studies have shown that inhibition of the mitochondrial calcium uniporter (MCU) protects DNs in pink1 mutant models, implicating calcium overload and ferroptosis in the PD. Many of these studies have explored PD using animal models. Zebrafish models offer advantages, including optical transparency, making them well-suited for in vivo studies. Current models and investigations predominantly focus on overt neurodegeneration, often overlooking the critical early phase of synaptic dysfunction.

AIM(S): Given the importance of synaptic dysfunction as an early pathological change in DNs—and the growing interest in using zebrafish models to study PD—our study aims to fill a critical gap by characterizing early physiological changes in PD zebrafish models.

METHOD(S): We aim to characterize the physiological properties of DNs in the periventricular posterior tuberculum (PPt)—a region implicated in PD—by applying local field potential (LFP) recording. This approach enables the detection of altered spike patterns and synaptic latency before the onset of cell loss. We will compare wild-type larvae with both transgenic PD models (pink1, lrrk2) and toxin-induced models (Erastin 5 µM, RSL3 10 µM, Iron 100 µM). Across these models, we will quantify spike amplitude, frequency, and power spectral density. Additionally, we will assess how these parameters change following treatment with the MCU inhibitor Ru360.

RESULTS: Our initial LFP recordings data show a higher frequency of downward spikes with greater amplitude in pink1 mutant 5dpf larvae compared to wild-type, suggesting altered synaptic excitability.

CONCLUSIONS: These findings may reveal early electrophysiological markers of Parkinson's disease and guide early intervention.

FINANCIAL SUPPORT: This study was supported by the National Science Centre (NCN), OPUS 25, project no. 2023/49/B/NZ4/02744 to JK.

P3.44. COMPARATIVE NEUROCHEMICAL AND SAFETY PROFILES OF PSILOCYBIN AND KETAMINE

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INTRODUCTION: Psilocybin and ketamine are fast-acting antidepressants, but their precise neurochemical actions and safety profiles differ. Both enhance monoaminergic and aminoacidergic neurotransmission but via distinct mechanisms.

AIM(S): To determine how psilocybin versus ketamine affect neurotransmitter release, molecular outcomes and rat behavior.

METHOD(S): Male Wistar rats were injected with psilocybin or ketamine. In vivo microdialysis examined extracellular DA, 5-HT, glutamate and GABA in cortical and limbic structures. Western blot was used to assess the expression of selected receptors. Locomotion, anxiety/depression-like behaviors and psychotogenic potential were tested using open-field, light–dark box, prepulse inhibition, head-twitch and forced swim tests.

RESULTS: Both compounds affected extracellular concentrations of examined neurotransmitters. High-dose psilocybin caused DNA damage in frontal cortex and hippocampus; ketamine affected only hippocampus. Neither drug produced clear antidepressant or anxiolytic behaviors. Low doses of psilocybin also elevated monoamines and GABA but did not cause DNA damage. Low-dose psilocybin produced an anxiolytic response without causing psychotomimetic effects.

CONCLUSIONS: Ketamine and psilocybin both acutely boost cortical monoamine and GABA levels, but their regional effects and safety differ. Importantly, high doses of psilocybin carried genotoxic risk in cortex and hippocampus that ketamine did not, whereas low doses of psilocybin were anxiolytic without toxicity. These results suggest that ketamine and psilocybin engage overlapping but distinct neurotransmitter systems

and receptors. Dose is critical: high doses of psilocybin require caution due to neurotoxicity, whereas low sub-psychedelic doses may offer anxiety relief safely.

FINANCIAL SUPPORT: Supported by the National Science Centre, Poland (grant no. 2020/37/B/NZ7/03753) and statutory funds of the Maj Institute of Pharmacology, Polish Academy of Sciences.

P3.45. LACK OF SORLA UNLOCKS THE PRO-INFLAMMATORY POTENTIAL OF GLIOMA-ASSOCIATED MICROGLIA

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INTRODUCTION: SorLA (SORL1 gene) is a sorting receptor which transports its protein cargoes between subcellular compartments and therefore defines their final localization. While SorLA was initially thought to be limited to neurons in the brain, it was later discovered to be expressed in glial cells as well, including microglia, which depending on the pathological context may acquire various functional properties. For instance, during glioblastoma (GBM) progression, both brain-resident microglia and blood-derived macrophages are reprogrammed, and instead of fighting the tumor, promote its growth through secretion of pro-tumorigenic factors. These cells are collectively called glioma associated microglia and macrophages (GAMs). Finding a mechanism responsible for the phenotypic polarization of GAMs is crucial for the development of new therapeutic strategies in the GBM treatment. Interestingly, our previous studies indicated that expression of SORL1 in human and mouse GAMs is upregulated [Kaminska et al., 2024].

AIM(S): To characterize the role of SorLA in shaping properties of GAMs.

METHOD(S): Primary mouse microglia were used in in vitro experiments. The impact of SorLA on glioma microenvironment was assessed in mouse GL261 gliomas implanted to wild-type (WT) and SorLA-deficient (SorLA-KO) mice.

RESULTS: We observed that the level of Sorl1 transcript depends on the activation mode of microglia. Also, lack of SorLA unlocks the ability of microglia to release higher amounts of pro-inflammatory factors when compared to WT cells. Furthermore, SorLA-KO microglia co-cultured with GL261 cells seem to exhibit enhanced phagocytosis and interferon-related responses. Our in vivo studies revealed that SorLA-KO mice develop smaller gliomas than the WT group, which coincides with pro-inflammatory activation of microglia and changes in the infiltration of immune cells from the periphery.

CONCLUSIONS: SorLA is a key player in shaping properties of microglia and its depletion unlocks their anti-tumor response, which prevents glioma growth.

FINANCIAL SUPPORT: Studies were supported by the National Science Center, Poland (2020/37/B/NZ3/00761, AM; 2023/49/N/NZ4/01690, PK) Foundation for Polish Science co–financed by the EU under the European Regional Development Fund (POIR.04.04.00 00 5CEF/18 00, AM) and I.3.4 Action of the Excellence Initiative – Research University Programme at the University of Warsaw.

P3.46. PROMOTING REMYELINATION IN THE CNS BY TARGETING NEUROINFLAMMATION

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INTRODUCTION: Persistent neuroinflammation is a major barrier to myelin repair in disorders such as multiple sclerosis (MS). We have investigated two distinct strategies—psychedelics and oxysterol-driven EBI2 activation—that modulate inflammatory signaling in the CNS and may independently support remyelination.

AIM(S): To evaluate (1) whether classical psychedelics can dampen inflammation-induced damage to oligodendrocytes and the blood–brain barrier (BBB), and (2) whether a synthetic EBI2 agonist promotes remyelination in a toxin model by altering glial inflammation and lipid profiles.

METHOD(S): Psychedelics: Organotypic mouse cerebellar slices were chemically demyelinated and treated with various psychodelics, alone or combined with ketanserin (5-HT_2A antagonist) or BD-1063 (Sigma-1

antagonist). In parallel, a human tri-cellular BBB model was exposed to pro-inflammatory stimuli with or without psychodelics and antagonists to assess the effects on barrier function. EBI2 agonist (CF_3-7α,25-OHC): Adult mice received cuprizone (CPZ) diet and then underwent two weeks of daily CF_3-7α,25-OHC injections.

RESULTS: Psychedelics: DMT reduced LPC-induced cytokine release and downregulated inflammatory transcription factors in both slice and BBB models. These effects were reversed by ketanserin or BD-1063, confirming 5-HT_2A and Sigma-1 receptor involvement in anti-inflammatory signaling. EBI2 agonist: CF_3-7α,25-OHC treatment accelerated MBP recovery in the corpus callosum. Treated mice exhibited increased levels of 15 lipid classes. Peripheral lymphocyte and monocyte counts decreased by ~50–60%, and brain Ebi2 transcripts rose significantly during remyelination.

CONCLUSIONS: Both approaches act on neuroinflammatory pathways—one via modulation of serotonin/Sigma-1 signaling, the other through oxysterol-EBI2–mediated effects on glial cells and lipids—to create a more favorable environment for myelin repair. These findings suggest that targeting neuroinflammatory signaling represents a promising therapeutic strategy for MS.

FINANCIAL SUPPORT: This project received funding from the National Science Centre, Poland, grant registration number: 2022/47/D/NZ3/02613.

P3.47. PSYCHEDELICS-MEDIATED NEUROIMMUNE SIGNALLING THROUGH 5-HT_2A AND SIGMA-1 RECEPTORS

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INTRODUCTION: Psychedelics are increasingly studied for their psychoactive properties, yet their direct cellular effects on neuroinflammation and neuroprotection remain poorly understood. These compounds are known to activate serotonin 5-HT_2A and/or Sigma-1 receptors, which play key roles in regulating neuronal survival, neuroplasticity and inflammatory signaling pathways. However, their potential involvement in promoting myelination or protecting against inflammation-induced demyelination has not been explored. Similarly, their effects on modulating blood–brain barrier (BBB) under normal and inflammatory conditions have yet to be investigated.

AIM(S): This project aims to determine whether psychedelics can protect against demyelination and neuron damage by activating anti-inflammatory signaling via 5-HT_2A and Sigma-1 receptors. In parallel, we will assess their effects on BBB integrity and permeability.

METHOD(S): Organotypic cerebellar slices were subjected to chemical demyelination and co-treated with DMT, either alone or in combination with selective antagonists: ketanserin (5-HT_2A receptor antagonist) and BD-1063 (Sigma-1 receptor antagonist). The release of pro- inflammatory cytokines and changes in gene expression were assessed by ELISAs and RT-qPCR. Human tri-cell BBB model was treated with psychedelics with or without a cocktail of pro-inflammatory cytokines IL17/TNFα and the antagonists (ketanserin or BD). Gene expression changes were assessed by RT-qPCR, including pro-inflammatory transcription factors, BBB components and the expression of 5-HT_2A and Sigma-1 receptors.

RESULTS: Treatment with psychedelics attenuated pro-inflammatory signaling in our ex vivo and in vitro models. The psychedelics-mediated anti-inflammatory effects were inhibited with receptor antagonists indicating 5-HT_2A and Sigma-1 receptors mediated effects.

CONCLUSIONS: This project and future findings may offer a cellular basis for exploring psychedelics as potential therapies in neuroimmune and neurodegenerative disorders.

FINANCIAL SUPPORT: No.

P3.48. FUNCTIONAL CONSEQUENCES OF INCREASED EBI2 SIGNALING IN GLIAL CELL BIOLOGY AND MYELINATION

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INTRODUCTION: Epstein-Barr virus-induced gene 2 (EBI2) and its oxysterol ligand 7α,25-dihydroxycholesterol (7α,25OHC) play a critical role in immune cell migration and have been implicated in neuroinflammatory and neurodegenerative diseases, including multiple sclerosis (MS). EBI2 is expressed in various immune cells such as B and T cells, dendritic cells, natural killer cells as well as in microglia, astrocytes and oligodendrocytes. In the CNS, EBI2 is upregulated in infiltrating glial cells. Astrocytes and microglia produce oxysterols

in response to inflammatory stimuli and facilitate astrocyte-macrophage communication. EBI2 is also transiently upregulated during oligodendrocyte maturation, and 7α,25OHC promotes oligodendrocyte progenitor cells (OPCs) migration, suggesting a role in myelination. High expression of EBI2 in immune cells and their accumulation in MS lesions supports the idea that the EBI2/7α,25OHC pathway facilitates the migration of inflammatory cells to sites of CNS inflammation in MS. These findings suggest EBI2 signaling is a key regulator of neuroimmune interactions and myelin biology, highlighting its therapeutic potential in demyelinating diseases such as MS.

AIM(S): This study will investigate the molecular and functional effects of increased EBI2 signaling in glial cells including astrocytes, microglia, and OPCs isolated from EBI2 knockout (KO) and WT mice and their role in myelination using organotypic cerebellar slice cultures.

METHOD(S): Cells will be treated with pro-inflammatory cytokines or lipopolysaccharide (LPS) with or without CF3-7α,25OHC to assess EBI2-dependent pathways. Functional outcomes in glial cells such as differentiation, maturation, and inflammatory responses will be evaluated using ELISA, qPCR, Western blot (WB), immunocytochemistry (ICC) along with (re)myelination in organotypic cerebellar slices.

RESULTS: Data collection and analysis are currently ongoing and preliminary findings will be available and presented at the conference.

FINANCIAL SUPPORT: The project received funding from the National Science Centre, Poland, grant registration number: 2022/47/D/NZ3/02613 (AR).

P3.49. THE SILENT NOCICEPTORS AS REGULATORS OF IMMUNE CELLS ACTIVITY

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INTRODUCTION: Tumors represent highly complex cellular assemblies, characterized by extensive vascularization and innervation. Recent evidence highlights a significant role of sympathetic and sensory innervation in promoting tumor progression. This has been attributed to the influence on immune cells – specifically through the release of the neuropeptide calcitonin gene-related peptide from nociceptive sensory neurons, leading to the cytotoxic T-cells exhaustion. Our recent work evidenced that this effect may be caused by silent nociceptors, a unique subpopulation of peptidergic nociceptors, which become "awakened" due to regulatory activity within the tumor niche.

AIM(S): To characterize interaction between silent nociceptors and the immune system in healthy transgenic mice.

METHOD(S): The study was conducted on ChRNA3-ERT2-Cre mice labelling silent nociceptors. Examination of the immune response was performed following selective opto- and chemogenetic modulation of silent nociceptors activity by high-throughput mass spectrometry and flow cytometry (FACS) of tibial bone marrow, as well as detailed immunohistochemistry and confocal microscopy analysis.

RESULTS: Immunohistochemical validation confirmed the effectiveness of optogenetic activation and chemogenetic block of silent nociceptors activity. Mass spectrometry analysis of mouse tibial marrow demonstrated the secretion of various neuropeptides by silent nociceptors known to influence immune system activity. FACS analysis confirmed significant alterations in the proportion of active and inactive cytotoxic T-cells in response to in vitro treatment with various neuropeptides and following silent nociceptors modulation in vivo.

CONCLUSIONS: The findings substantiate significant impact of silent nociceptors activity on immune system function. The opto- and chemogenetic modulation provides a robust experimental framework to facilitate future phenotyping in a context of bone tumors. The data acquired herein provide a valuable and necessary basis for these prospective studies.

FINANCIAL SUPPORT: Funded by The Polish National Agency for Academic Exchange Strategic Partnerships Grant (BNI/PST/2023/1/00132/U/00001), held by M. Kucharczyk.

P3.50. GPR84 MODULATES INFLAMMATORY SIGNALING IN NEUTROPHILS AND MICROGLIA: A DUAL-CELLULAR APPROACH TO CHRONIC PAIN MECHANISMS

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INTRODUCTION: Chronic pain involves complex neuroimmune interactions, yet molecular drivers of inflammation across peripheral and central immune cells are poorly understood. GPR84, a pro-inflammatory GPCR expressed in myeloid cells, is a potential regulator of such responses.

AIM(S): To elucidate the role of GPR84 in driving inflammatory responses in both neutrophil-like and microglial cells, with a focus on its potential as a shared therapeutic target in peripheral and central pain mechanisms.

METHOD(S): NB4 cells were differentiated with ATRA and treated with DL175, LPS, NEL1, or their combinations (DL175+LPS, DL175+NEL1, LPS+NEL1). CyTOF was performed on days 3, 5, and 7 to assess activation and maturation markers. HMC3 cells were treated with DL175, LPS, STS, NEL1, nigericin, and their combinations (DL175+LPS, DL175+LPS+Nigericin, STS+DL175, NEL1+LPS, NEL1+LPS+Nigericin, STS+NEL1). RT-qPCR assessed expression of IL1B, IL18, NLRP3, CASP1, CASP4, NF-κB, GPR84.

RESULTS: CyTOF revealed that GPR84 stimulation promoted neutrophil maturation (CD11b, CD66b, CD44) and enhanced co-stimulatory molecule expression (HLA-DR), particularly under LPS co-treatment. Antagonist application attenuated these effects, confirming receptor specificity. In HMC3, DL175 amplified LPS- and STS-induced transcription of NLRP3, IL1, CASP1/4, and NF-κB. The DL175+LPS+nigericin condition showed the strongest IL18 and NLRP3 upregulation. Co-treatment with antagonist attenuated these effects, confirming receptor specificity.

CONCLUSIONS: GPR84 drives pro-inflammatory polarization in neutrophils and microglia. It may represent a shared therapeutic target in chronic pain-related immune responses.

P3.51. FROM GENES TO ACTIVITY: INTEGRATED ANALYSIS OF PROTEASOME FUNCTION IN MICROGLIA AND BRAIN REGIONS IN NEUROPATHIC PAIN

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INTRODUCTION: Neuropathic pain(NP) targets 7-10% of population, however only 30-40% of patients experience relief from the available treatments and a look for new molecular targets remains an urgent challenge. The nerve injuries lead to the release of TLR-4 agonists triggering the activation of NF-kB, which leads to the transcription of proinflammatory cytokines. The mechanism is regulated by the degradation via constitutive (c20S) and immuno-(i20S) proteasome.

AIM(S): The aim was to determine the expression levels of β subunits of c20S/i20S in murine Chronic Constriction Injury (CCI) model adopting a multiomic approach.

METHOD(S): We employed the multiomic approach by simultaneous analysis of transcriptome, proteome and single-cell proteome via RT-qPCR, Western Blot, and IMC via Hyperion System within 6 CNS regions.

RESULTS: Multilevel profiling shows that proteasome expression in NP is highly region-specific. Only the ipsilateral spinal cord displays a full inflammatory switch, exhibiting parallel upregulation of β1/β5 and LMP2/LMP7. On the other hand, remaining structures favor hybrid cores in which MECL-1 replaces β2 without full induction. IMC analysis of spinal cord further revealed that LMP2 and LMP7 are differentially expressed across distinct cell clusters. LMP2 expression was enriched in cluster 10, co-expressing IL-17RA and TLR4, suggesting association with inflammatory microglia. LMP7 showed upregulation in cluster 1, alongside GFAP, indicating involvement of astrocytes.

CONCLUSIONS: We demonstrate that i20S and c20S expression in neuropathic pain is region-specific within the CNS. The ipsilateral spinal cord emerges as the principal neuroinflammatory site, whereas the remaining regions display adaptive signatures of heightened proteotoxic stress. The formation of hybrid catalytic cores containing MECL-1 in supraspinal regions led to identifying MECL-1 as a putative regulator of the chronic phase. These findings argue for site and subunit-directed modulation of the proteasome rather than pan-complex inhibition.

FINANCIAL SUPPORT: The project was funded by the National Science Centre (OPUS grant no. 2023/49/B/NZ7/02172).

P3.52. IN VIVO INVESTIGATION OF THE SEX-DEPENDENT ANALGESIC EFFECTS OF HISTAMINE H4 RECEPTOR ANTAGONIST IN A MURINE MODEL OF NEUROPATHIC PAIN

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INTRODUCTION: Neuropathic pain is a complex condition significantly modulated by neuroimmune interactions. The histamine H4 receptor (H4R) has emerged as a promising target for pharmacological intervention, with its expression strongly associated with immune responses.

AIM(S): This study aimed to investigate the mechanism of action of a novel H4R antagonist (JSJ; 5-chloro-1H-indol-2-yl)(4-methylpiperazin-1-yl)methanethione hydrochloride) in neuropathic male and female mice, and its influence on spinal astrocyte activation.

METHOD(S): We assessed the effects of JSJ on mechanical (von Frey) and thermal (cold plate, tail flick) stimuli in a chronic constriction injury (CCI) model in both sexes. Single (1, 10, 20 mg/kg, i.p.) and repeated (20 mg/kg, i.p., twice daily for 7 days) JSJ administrations were evaluated. H4R expression and JSJ's impact on astrocyte activation (GFAP level) were examined using immunohistochemistry and Western blotting, respectively.

RESULTS: A single JSJ injection attenuated thermal hyperalgesia in neuropathic females. In contrast, repeated administration significantly reduced mechanical allodynia in CCI-exposed males. Biochemical analyses revealed nerve injury-induced upregulation of GFAP in males, but not in females. JSJ treatment did not significantly affect GFAP levels. H4R expression was confirmed in spinal astrocytes in both sexes.

CONCLUSIONS: These findings underscore sex-dependent differences in the analgesic effects of H4R antagonism, potentially mediated by divergent astrocytic activation profiles.

FINANCIAL SUPPORT: This work was financed by a grant from the National Science Centre, Poland, SONATA 2019/35/D/NZ7/01042.

P3.53. SEX-DEPENDENT DIFFERENCES IN NEUROPATHIC PAIN: INSIGHTS FROM FUNCTIONAL METABOLIC PHENOTYPING AND IMMUNOLOGICAL ALTERATIONS

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INTRODUCTION: Sex is recognized as a significant biological variable in chronic neuropathic pain. Emerging evidence suggests that neuroimmune interactions contribute substantially to sex-related differences in pain mechanisms.

AIM(S): The aim of this study was to identify sex-dependent differences in metabolic and immunological responses in the spinal cord of neuropathic mice.

METHOD(S): Neuropathic pain was induced via chronic constriction injury (CCI) of the sciatic nerve in male and female mice. Fourteen days post-surgery, animals were monitored in Promethion metabolic cages for 24 hours. Parameters assessed included oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER), energy expenditure (EE), and locomotor activity. Spinal cord immunoprofiles were evaluated using the Hyperion Imaging System.

RESULTS: Neuropathic males exhibited a reduced RER compared to naïve controls, while no change was observed in females. Females showed lower EE in both naïve and CCI groups. Furthermore, CCI females displayed decreased sleep time compared to naïve. Locomotor activity remained unchanged across all groups. Preliminary immunological analyses indicated reduced GFAP (astrocyte marker) expression in CCI females, whereas CCI males exhibited increased GFAP levels. IBA1 (microglial marker) levels were elevated in both sexes following CCI.

CONCLUSIONS: These data reveal sex-dependent differences in metabolic and immunological responses in neuropathic mice, emphasizing the necessity for sex-specific approaches in the development of pain therapies.

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AUTHOR INDEX

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