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PROGRAMME

27.08.2019, TUESDAY

12:00–14:00 **Workshop on Ultrasonic Vocalization (USV) - theory**

14:00–15:00 **Lunch**

15:00–18:00 **Workshop on Ultrasonic Vocalization (USV) - practice**

28.08.2019, WEDNESDAY

12:00–14:30 **General Assembly of PTBUN**

14:30–15:00 **Opening Ceremony**

15:00–16:00 **Plenary lecture 1 (PL 1)**
Speaker: Magdalena Götz, Helmholtz Center Munich, German Research Center for Environmental Health, Munich, Germany
Title: "Novel mechanisms of neurogenesis and neural repair"
Introduced by: Jarosław J. Barski

16:00–16:30 **Coffee break**

16:30–17:30 **Plenary lecture 2 (PL 2)**
Speaker: Hannah Monyer, German Cancer Research Center, Heidelberg, Germany
Title: "GABAergic neurones – the cellular substrate for local and long-range synchrony"
Introduced by: Jarosław J. Barski

17:40–19:40 **Symposium 1: "Ultrasonic vocalization as a tool in neuroscience research"**

Chairperson:
- Paweł M. Boguszewski, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Speakers:
- Nicola Simola, Department of Biomedical Sciences, University of Cagliari, Italy,
"Emission of 50-kHz ultrasonic vocalizations in dopamine-denervated rats treated with amphetamine: relevance to neurocircuitries involved in drug-mediated reward" (S 1.1)

- Sylvie Granon, Paris-Saclay Institute of Neuroscience, Orsay, France,
"Social interaction and acoustic communication in adult mice: markers for healthy and pathological behaviors" (S 1.2)

- Robert K. Filipkowski, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland,
"Behavioral, ultrasonic, and cardiovascular responses of male Wistar rats in different social and emotional contexts after ultrasonic playback" (S 1.3)

- Adam Hamed, Laboratory of Spatial Memory, Department of Cellular and Molecular Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
"Neurochemical correlates of 50-kHz ultrasonic vocalizations in context induced response, reward processing, and appetitive social interactions" (S 1.4)

Symposium 2: "Relationship between mitochondria and neuroinflammation: implications for neurological diseases"

Chairpersons:
- Agata Adamczyk, Mossakowski Medical Research Centre, Polish Academy of Sciences
- Carsten Culmsee, Philipps-Universität Marburg, Germany

Speakers:
- Agnieszka Chacińska, Centre of New Technologies, University of Warsaw, Poland,
"Guided tour of proteins into mitochondria" (S 2.1)

- Carsten Culmsee, Institute of Pharmacology and Clinical Pharmacy, Philipps-Universität Marburg, Germany,
"Mitochondrial integrity and function in model systems of CNS diseases" (S 2.2)

- Katarzyna Kuter, Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland,
"Brain energy metabolism in neurodegeneration of dopaminergic neurons in animal model of early Parkinson's disease: Role of astrocytes" (S 2.3)

- Magdalena Cieślik, Department of Cellular Signalling, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland,
"The association between maternal immune activation and mitochondrial failure in adulthood: Relevance to neurodevelopmental disorders" (S 2.4)

- Walter Lukiw, LSU Neuroscience Center, New Orleans, USA,
"Gastrointestinal (GI) tract-derived, Bacteroidetes fragilis neurotoxins and inflammatory neurodegeneration" (S 2.5)

Symposium 3: "Whole brain mapping approaches in the investigation of brain structure and function: advances, promises, and challenges"

Chairperson:
- Piotr Majka, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Speakers:
- Piotr Majka, Laboratory of Neuroinformatics, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
"Location, location, location: The role of brain atlases and spatial integration of multimodal imaging data in whole brain mapping projects" (S 3.1)

- Marzena Stefaniuk, Laboratory of Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
"Addicted brain mapping and imaging using light-sheet fluorescence microscopy" (S 3.2)

- Yongsoo Kim, Department of Neural and Behavioral Sciences, College of Medicine, Penn State University, Hershey, Philadelphia, USA,
"Brain-wide mapping of oxytocin receptor neurons in the developing postnatal mouse brain" (S 3.3)

- Marcello Rosa, Biomedicine Discovery Institute and Department of Physiology, Monash University, Melbourne, Australia,
"An open access tool for analysis of connections in the primate cortex" (S 3.4)

19:40– **Welcome reception**

29.08.2019, THURSDAY

08:30–09:30 **Plenary lecture 3 (PL 3)**
Speaker: Hartmut Wekerle, Max Planck Institute of Neurobiology, Martinsried, Germany
Title: "Multiple sclerosis: Formation of a brain autoimmune disease"
Introduced by: Jarosław J. Barski

09:30–09:45 **Coffee break**

09:45–11:45 **Symposium 4: "Modeling human disorders in Zebrafish"**

Chairpersons:
- Jacek Kuźnicki, International Institute of Molecular and Cell Biology, Warsaw, Poland
- Justyna Zmorzyńska, International Institute of Molecular and Cell Biology, Warsaw, Poland

Speakers:
- Piotr Podlasz, Department of Pathophysiology Forensic Veterinary Medicine and Administration, Faculty of Veterinary Medicine, University of Warmia i Mazury, Olsztyn, Poland,
"Study of neuropeptide functions using zebrafish as a model organism" (S 4.1)

- Caghan Kizil, German Center for Neurodegenerative Diseases, Dresden, Germany,
"Identification of a novel mechanism controlling neural stem cell plasticity in an Alzheimer's disease model of adult zebrafish using single cell transcriptomics" (S 4.2)

- Justyna Zmorzyńska, Laboratory of Molecular and Cellular Neurobiology, International Institute of Molecular and Cell Biology, Warsaw, Poland,
"Impaired commissural tract fasciculation is related to increased epileptogenesis and anxiety in the Zebrafish model of Tuberous Sclerosis Complex" (S 4.3)

- Allan V. Kalueff, School of Pharmacy, Southwest University, Chongqing, China,
"How zebrafish research is reshaping today's neuroscience and biological psychiatry" (S 4.4)

Symposium 5: "Nutrition and brain"

Chairperson:
- Jarosław J. Barski, Department for Experimental Medicine, Medical University of Silesia, Katowice, Poland

Speakers:
- Marta Nowacka-Chmielewska, Department for Experimental Medicine, Medical University of Silesia, The Jerzy Kukuczka Academy of Physical Education, Katowice, Poland,
"Global proteomic analysis of brain changes induced by 'lifestyle' modifications" (S 5.1)

- Witold Konopka, Laboratory of Animal Models, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
"Why and what do we eat or brain-body metabolic games" (S 5.2)

- André Kleinridders, Central Regulation of Metabolism, German Institute of Human Nutrition, Nuthetal, Germany,
"Novel insights of brain insulin action on metabolism and behavior" (S 5.3)

- Claire T. McEvoy, Institute for Global Food Security, Queen's University Belfast, Northern Ireland,
"Diet as a prevention strategy for neurodegeneration during ageing" (S 5.4)

Symposium 6: "Basic and clinical aspects of epilepsy research"

Chairperson: • Piotr Suffczyński, Faculty of Physics, University of Warsaw, Poland

Speakers: • Marco de Curtis, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy,
"Network activities at the start of a focal seizure" (S 6.1)

• Premysl Jiruska, Institute of Physiology, The Czech Academy of Sciences, Prague, Czech Republic,
"The transition to seizure is characterized by the progressive loss of the brain's resilience" (S 6.2)

• Urszula Malinowska, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
"Pathological synchronization: biomarkers and networks in human epilepsy" (S 6.3)

• Piotr Suffczyński, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Poland,
"Neuronal and ionic mechanisms of focal seizures – insight from an in silico study" (S 6.4)

11:55–12:40 **Special Lecture 1: Konorski Award presentation, Anna Beroun**
Marzena Stefaniuk, Anna Beroun, Tomasz Lebitko, Olga Markina, Szymon Łęski, Ksenia Meyza, Anna Grzywacz, Jerzy Samochowiec, Agnieszka Samochowiec, Katarzyna Radwańska and Leszek Kaczmarek
"Matrix Metalloproteinase-9 and Synaptic Plasticity in the Central Amygdala in Control of Alcohol-Seeking Behavior"

12:40–13:40 **Lunch**

13:20–13:40 **Wykład sponsora SHIM-POL**
• Marek Szklarczyk,
"Zastosowanie techniki fNIRS do obrazowania czynności mózgu" (in Polish)

13:40–15:40 **Symposium 7: "Posttranscriptional modifications of gene expression and central nervous system pathologies"**

Chairperson: • Adrian Smędowski, Chair and Department of Physiology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

Speakers: • Marialaura Amadio, Department of Drug Sciences, Section of Pharmacology, University of Pavia, Pavia, Italy,
"ELAV proteins and neurodegeneration: what is the role in Alzheimer's disease?" (S 7.1)

• Alessandro Quattrone, Laboratory of Translational Genomics, Centre for Integrative Biology, University of Trento, Trento, Italy,
"Elav proteins in the pathogenesis of motoneuron diseases" (S 7.2)

• Adrian Smędowski, Chair and Department of Physiology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland,
"Increased intraocular pressure alters the cellular distribution of HuR protein in retinal ganglion cells" (S 7.3)

• Marita Pietrucha-Dutczak, Chair and Department of Physiology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland,
"Organotypic retinal explants model for evaluation of neurotoxicity and neuroprotection – the impact of metallothionein treatment on HuR protein content" (S 7.4)

Symposium 8: "Mechanisms of GABAergic plasticity"

Chairperson: • Jerzy W. Mozrzymas, Department of Biophysics, Wroclaw Medical University, Wroclaw, Poland

Speakers: • Enrico Cherubini, European Brain Research Institute, Rome, Italy,
"Impairment of synaptic plasticity at immature GABAergic mossy fiber-CA3 synapses in animal models of Autism" (S 8.1)

• Andrea Barberis, Italian Institute of Technology, Genoa, Italy,
"Spatial regulation of coordinated excitatory and inhibitory synaptic plasticity at dendritic synapses" (S 8.2)

• Joanna Urban-Ciećko, Laboratory of Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
"Learning-evoked modulation of GABAergic interneurons intrinsic excitability in the neocortex" (S 8.3)

• Jerzy W. Mozrzymas, Department of Biophysics, Wroclaw Medical University, Wroclaw, Poland,
"GABAergic hippocampal plasticity critically depends on Matrix metalloproteinase 3" (S 8.4)

Symposium 9: "miRNAs role in epilepsy – potential diagnostic and therapeutic applications"

Chairperson: • Kinga Szydłowska, Laboratory of Epileptogenesis, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
Co-chair: • Katarzyna Łukasiuk

Speakers: • Sergiusz Jóźwiak, Department of Child Neurology, Medical University of Warsaw, Warsaw, Poland,
"Molecular biomarkers of epileptogenesis in a genetic model of epilepsy – tuberus sclerosis complex" (S 9.1)

• Ervin van Vliet, Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, Amsterdam, The Netherlands,
"MicroRNAs in experimental and human temporal lobe epilepsy: biomarker and therapeutic potential" (S 9.2)

• Noora Puhakka, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland,
"MircoRNAs in experimental post-traumatic epilepsy: biomarker and therapeutic potential" (S 9.3)

• Kinga Szydłowska, Laboratory of Epileptogenesis, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
"Circulating microRNAs as a biomarker of epileptogenesis/epilepsy" (S 9.4)

15:50–16:40 **Special lecture 2: Flatau Award presentation, Leszek Kaczmarek**

16:40–18:40 **Poster Session 1 / Coffee + Meet the speakers**

16:40–17:00 **Wykład sponsora OMIXYS Sp. z o.o.**
Paula Perin, Field Application Scientist for Europe,
"Ultra-sensitive detection of biomarkers with Simoa technology to understand neurodegenerative diseases"

17:00–18:30 **Oferta konkursowa Narodowego Centrum Nauki dla osób rozpoczynających karierę naukową (Grant opportunities for young scientists)**
Małgorzata Hasiec, Narodowe Centrum Nauki

18:45–19:45 **Plenary lecture 4 (PL 4)**
Speaker: Marcello Rosa, Biomedicine Discovery Institute and Department of Physiology, Monash University, Melbourne, Australia
Title: "Plasticity of the primate visual pathway following lesions in early and mature life"
Introduced by: Piotr Majka

20:30– **Get Together Party**

30.08.2019, FRIDAY

08:30–09:30 **Plenary lecture 5 (PL 5)**
Speaker: Gernot Riedel, The Institute of Medical Sciences, University of Aberdeen, King's College, Aberdeen, UK
Title: "Preclinical development of treatments for tauopathies: translation to the clinic and back"
Introduced by: Jarosław J. Barski

09:30–09:45 **Coffee break**

09:45–11:45 **Symposium 10: "New methods in motor control"**

Chairperson:
- Hanna Drzymała-Celichowska, Department of Neurobiology / Department of Biochemistry, Poznan University of Physical Education, Poznan, Poland

Speakers:
- Marin Manuel, French National Centre for Scientific Research, Paris, France,
"Electrophysiological properties of functionally identified adult mouse motoneurons" (S 10.1)

- Francesco Negro, Department of Clinical and Experimental Sciences, University of Brescia, Italy,
"Estimating the transfer function of rat muscle units" (S 10.2)

- Piotr Kaczmarek, Institute of Control, Robotics and Information Engineering at Poznan University of Technology, Poznan, Poland,
"The mechanomyogram - a valuable tool to analyze a mechanical activity of the muscle" (S 10.3)

- Hanna Drzymała-Celichowska, Department of Neurobiology / Department of Biochemistry, Poznan University of Physical Education, Poznan, Poland,
"Sag in motor unit unfused tetanus: extra force production at the beginning of activity" (S 10.4)

Symposium 11: "Dopamine neurons: from neuronal activity to behavioral outcomes"

Chairpersons:
- Tomasz Błasiak, Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland
- Jan Rodriguez Parkitna, Department of Molecular Neuropharmacology Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

Speakers:
- Tomasz Błasiak, Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland,
"How certain are certain 'certainties' about DA neurons?" (S 11.1)

• Boris Gutkin, Mathematics of Neural Circuits, LNC2, École Normale Supérieure, Paris, France and Institute for Cognitive Neuroscience, NRU Higher School of Economics, Moscow, Russia,
"Using computational models to understand endogenous and exogenous control over dopamine cell activity: when inhibition leads to bursting" (S 11.2)

• David Engblom, Department of Clinical and Experimental Medicine, Center for Social and Affective Neuroscience, Linköping University, Linköping, Sweden,
"Sickness and mood: inflammatory modulation of dopamine signaling" (S 11.3)

• Bradley M. Roberts, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK and Oxford Parkinson's Disease Centre, Oxford, UK,
"Striatal GABA transporter activity governs dopamine transmission and shows maladaptive downregulation in a mouse model of parkinsonism" (S 11.4)

Symposium 12: "The role of Arc/Aerg3.1 in neuronal plasticity"

Chairperson: • Katarzyna Radwańska, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Speakers: • Hiroyuki Okuno, Department of Biochemistry and Molecular Biology, Kagoshima University School of Medicine,
"Control of AMPA receptor dynamics at active and inactive synapses and of cognitive refinement by Arc/Arg3.1" (S 12.1)

• Katarzyna Radwańska, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,
"The role of Arc/Arg3.1 protein in the regulation of alcohol seeking" (S 12.2)

• Jacek Jaworski and Agata Góźdź, International Institute of Molecular and Cell Biology, Warsaw, Poland,
"Role of Arc phosphorylation in structural plasticity" (S 12.3)

• Sonia Correa, Faculty of Life Sciences, University of Bradford, UK,
"The temporal dynamics of Arc expression regulate cognitive flexibility" (S 12.4)

11:55–12:40 **Special lecture 3: Young Investigator Award**

12:40–13:40 **Lunch**

13:40–15:40 **Poster session 2/Coffee + Meet the speakers**

14:00–15:30 **Oferta konkursowa Narodowego Centrum Nauki dla doświadczonych naukowców (Grant opportunities for experienced scientists)**
Małgorzata Hasiec, Narodowe Centrum Nauki

15:40–16:40 **Plenary lecture 6 (PL 6)**
Speaker: Graham Sheridan, School of Pharmacy and Biomolecular Sciences, Centre for Stress and Age-Related Disease, University of Brighton, Brighton, UK
Title: "Mechanobiology of the brain in health and disease"
Introduced by: Aleksandra Rutkowska

16:40–17:10 **Closing ceremony**

17:10–17:40 **PTBUN Governing Council Meeting**

POSTER SESSION I

P1 – DISEASE MODELS I

P1.1 **Prenatal exposure to valproic acid leads to behavioral alterations and defects of synaptic proteins in the hippocampus of adolescent rats**
Agata Adamczyk, Magdalena Gąssowska-Dobrowolska, Magdalena Cieślik, Grzegorz A. Czapski, Henryk Jęśko, Magdalena Gewartowska, Małgorzata Frontczak-Baniewicz

P1.2 **Prenatal exposure to valproic acid leads to deregulation of purinergic signaling in the brain of adolescent rats**
Lidia Babiec, Anna Wilkaniec, Marta Matuszewska, Wojciech Hilgier, Agata Adamczyk

P1.3 **Evaluation of transgenic mice with progressive degeneration of noradrenergic system towards the study of presymptomatic phase of Parkinson's Disease**
Justyna Barut, Piotr Chmielarz, Agnieszka Jurga, Monika Bagińska, Rosanna Parlato, Andrii Domanskyi, Irena Nalepa, Grzegorz Kreiner

P1.4 **Modification of monosynaptic Ia EPSP by externally induced electrical fields in SOD1 G93A mouse model of amyotrophic lateral sclerosis (ALS)**
Marcin Bączyk, Tomasz Jankowiak, Marcin Cholewiński

P1.5 **Modulation of hippocampal theta activity by transcutaneous stimulation of trigeminal nerve in anesthetized rats**
Renata Bocian, Adam Broncel, Paulina Kłos-Wojtczak, Jan Konopacki

P1.6 **Neuroprotective effects of metabotropic glutamate receptors group II (mGluR2/3) agonists in an animal model of birth asphyxia**
Ewelina Bratek, Apolonia Ziembowicz, Elżbieta Salińska

P1.7 **Maternal separation modifies dendritic spine density in ventral tegmental area neurons in a sub-region-specific manner: study in female rats**
Aleksandra Celary, Anna Guguła, Grzegorz Tylko, Grzegorz Hess, Anna Błasiak

P1.8 **Comparison of dopaminergic neurons generated from iPS cells from healthy volunteers and patients with the idiopathic form of Parkinson's disease (PD)**
Paula Chlebanowska, Anna Tejchman, Maciej Sułkowski, Marcin Majka

P1.9 **DAT1 gene, ADHD, and the development of attentional functions**
Anita Cybulska-Kłosowicz, Katarzyna Kuć, Maksymilian Bielecki, Ewa Racicka-Pawlukiewicz, Michał B. Czerwiński

P1.10 **CacyBP/SIP and β-catenin homeostasis in the YAC128 mice model of Huntington's disease**
Magdalena Czeredys, Ewelina Latoszek, Jan Ludwiczak, Stanisław Dunin-Horkawicz, Jacek Kuźnicki

P1.11 **Traumatic brain injury-induced changes in electrophysiological properties of granule cells and adult hippocampal neurogenesis**
Joanna Danielewicz, Irene Dura, Juan Manuel Encinas

P1.12 **Lipopolysaccharide accelerates neuroinflammation in a mouse model of Alzheimer's disease**
Jan Długosz, Angelika Więckowska, Maciej Koperski, Anna Mietelska-Porowska, Urszula Wojda

P1.13 **Diet-derived changes in insulin metabolism in brain accelerate the development of Alzheimer's disease neuropathological features**
Anna Mietelska-Porowska, Angelika Więckowska, Jan Długosz, Maciej Koperski, Urszula Wojda

P1.14 **Western diet – from metabolic disturbances to acceleration of glia activation and development of Alzheimer's disease**
Angelika Więckowska, Anna Mietelska-Porowska, Małgorzata Wydrych, Jan Długosz, Dominik Chutorański, Urszula Wojda

P1.15 **Identification of potential Keap1 inhibitors through database analysis based on the designated pharmacophore and QSAR models**
Roksana Duszkiewicz, Justyna Gacek

P1.16 **Subthalamic nucleus deep brain stimulation influence on erythrocytes number and hemoglobin levels in a rat model of early Parkinson's disease**
Beata Grembecka, Paulina Koriat, Magdalena Podlacha, Irena Majkutewicz, Kacper Ptaszek, Karolina Plucińska, Danuta Wrona

P1.17 **Social play behavior of rats in the model of autism induced by prenatal exposure to polyinosinic: polycytidylic acid**
Kinga Gzieło, Małgorzata Hołuj, Piotr Popik, Agnieszka Nikiforuk

P1.18 **Phase-amplitude cross-frequency coupling in rat's olfactory bulb after injection of ketamine**
Gabriela Jurkiewicz, Mark J. Hunt , Jarosław Żygierewicz

P1.19 **Increased anxiety-related behavior in a zebrafish model of Tuberous Sclerosis Complex recapitulated human symptoms of the disease**
Magdalena Kędra

P1.20 **The effect of transcutaneous stimulation of the vagus nerve on hippocampal formation theta rhythm in anaesthetized rats**
Jan Konopacki, Adam Broncel, Renata Bocian, Paulina Kłos-Wojtczak

P2 – MOTOR SYSTEMS

P2.1 **The role of drebrin in the neuromuscular junction**
Paloma Alvarez-Suarez, Marta Gawor, Tomasz J. Prószyński

P2.2 **What planning interactions with tools can tell us about bimanuality: an fMRI study**
Mikołaj Buchwald, Agnieszka Nowik, Grzegorz Króliczak

P2.3 **The number and motor innervation of muscle spindles in the medial gastrocnemius of male and female rats**
Jan Celichowski, Magdalena Gartych, Barbara Mierzejewska-Krzyżowska, Dorota Bukowska

P2.4 **Long-lasting effects of trans-spinal direct current stimulation on motoneuron firing properties in rats**
Piotr Krutki, Hanna Drzymała-Celichowska, Włodzimierz Mrówczyński, Marcin Bączyk

P2.5 **The influence of endurance, strength, and vibration training on sag in unfused tetanic contractions of fast motor units**
Katarzyna Kryściak, Hanna Drzymała-Celichowska, Ian Curtis Smith, Jan Celichowski

P2.6 **Hypothermia induced changes in contractile properties of motor units in rat medial gastrocnemius**
Bartosz Malak, Jan Celichowski, Hanna Drzymała-Celichowska

P2.7 **Neural bases of actions involving complex motor-to-mechanical transformations**
Maciej Raś, Michał Wyrwa, Grzegorz Króliczak

P2.8 **The role of Amot and Yap1 in the regulation of dendritic tree morphogenesis and cerebellar functions**
Katarzyna Rojek, Joanna Krzemień, Hubert Doleżyczek, Marcin Rylski, Leszek Kaczmarek, Jacek Jaworski, Tomasz J. Prószyński

P2.9 **Effect of a 4-week mental training on decrease in grasping force in young healthy people**
Katarzyna Kisiel-Sajewicz, Magdalena Siemiatycka, Joanna Mencel, Łukasz Kamiński, Jarosław Marusiak, Artur Jaskólski, Anna Jaskólska

P2.10 **Effect of motor imagery training of reaching-to-grasp on cortical activity related to motor imagery and motor execution of grasping by dominant hand in healthy people**
Joanna Mencel, Łukasz Kamiński, Jarosław Marusiak, Anna Jaskólska, Artur Jaskólski, Katarzyna Kisiel-Sajewicz

P3 – LEARNING AND BEHAVIOR I

P3.1 **Evaluation of spatial generalization in house cricket (*Acheta domesticus*)**
Bartosz Baran, Michał Krzyżowski, Jacek Francikowski, Mateusz Hohol

P3.2 **Ketogenic diet and sexual motivation in male rats**
Wiktor Bogacki-Rychlik

P3.3 **Fish as animal models in biomedical research**
Dominika Chojnacka, Jarosław J. Barski

P3.4 **Social interactions and ultrasonic vocalisation in serotonin transporter knockout rats**
Joanna Gołębiowska, Małgorzata Hołuj, Diana Piotrowska, Agnieszka Nikiforuk, Piotr Popik, Judith Homberg

P3.5 **Presence of a companion alleviates fear response but does not equal fear extinction**
Tomasz Górkiewicz, Karolina Rokosz-Andraka, Kacper Kondrakiewicz, Ksenia Meyza, Ewelina Knapska

P3.6 **The influence of fatty-acid amide hydrolase inhibitors on memory-related behaviors provoked by the cholinergic receptor ligands in mice**
Marta Kruk-Słomka, Agnieszka Dzik, Tomasz Słomka, Grażyna Biała

P3.7 **Amotl1: a novel synaptic protein important for mice social behavior and the brain organization**
Joanna Krzemień, Katarzyna Rojek, Maciej Winiarski, Paweł M. Boguszewski, Ewelina Knapska, Tomasz J. Prószyński

P3.8 **Time-related discriminatory stimulus following LSD administration in rats**
Agata Kuziak, Martyna Krawczyk, Agnieszka Nikiforuk, Piotr Popik

P3.9 **A test for assessing prosocial behavior in mice**
Klaudia Misiołek, Zofia Harda, Bartosz Baran, Michał Krzyżowski, Jan Rodriguez Parkitna

P4 – SENSORY SYSTEMS

P4.1 **UV light detection by the rat olivary pretectal nucleus**
Anna Alwani

P4.2 **Chemogenetic inhibition of somatostatin interneurons alters plasticity induced by sensory deprivation in the barrel cortex of mice**
Grzegorz Dobrzański, Agnieszka Łukomska, Renata Zakrzewska, Małgorzata Kossut

P4.3 **Kcnb1 plays a role in the development and function of the ear in zebrafish**
Justyna Jędrychowska, Evgeny Gasanov, Jacek Kuźnicki, Vladimir Korzh

P4.4 **UV light detection by the rat dorsal lateral geniculate nucleus**
Anna Kustron, Jagoda Jęczmień-Łazur, Patrycja Orlowska-Feuer, Marian H. Lewandowski

P4.5 **Experience-dependent acquisition of visuomotor behavioral representation in primary visual cortex**
Alicja Puścian, Hadas Benisty, Lan Tang, Michael J. Higley

P5 – NEUROSCIENCE METHODS

P5.1 **Whole-brain mapping of neuroplasticity in different experimental paradigms in mice - a computational perspective**
Sylwia Bednarek, Natalia Jermakow, Marzena Stefaniuk, Monika Pawłowska, Daniel K. Wójcik, Piotr Majka

P5.2 **Exploratory and classical ANOVA analysis of response-locked event-related potentials (ERPs)**
Joanna Duda-Goławska, Kamil K. Imbir, Jarosław Żygierewicz

P5.3 **Microelectronic system for low-artifact electrical stimulation and recording of brain activity at up to 512 electrodes**
Małgorzata Szypulska, Piotr Wiącek, Andrzej Skoczeń, Imran Ahmed, Tomasz Fiutowski, Paweł Jurgielewicz, Karolina Kołodziej, Michał B. Czerwiński, Daniel K. Wójcik, Bartosz Mindur, Ewa Kublik, Władysław Dąbrowski, Paweł Hottowy

P5.4 **Intracellular recording of mouse spinal motoneurons in vivo**
Tomasz Jankowiak, Marcin Cholewiński, Marcin Bączyk

P5.5 **PyEcoHAB: a Python library for analysis of rodent behavioral data recorded with Eco-HAB**
Joanna Jędrzejewska-Szmek, Jan Mąka, Szymon Łęski, Maciej Winiarski, Daniel K. Wójcik, Ewelina Knapska

P5.6 **Kernel Electrical Source Imaging (kESI) method for reconstruction of sources of brain electric activity in realistic brain geometries**
Marta Kowalska, Jakub M. Dzik, Chaitanya Chintaluri, Daniel K. Wójcik

P5.7 **Assessment of biomarkers of attention in modified delay match-to-sample test**
Marek Waligóra, Urszula Malinowska, Andrzej Wróbel, Jakub Wojciechowski, Jacek Rogala

P5.8 **Kernel current source density revisited**
Chaitanya Chintaluri, Marta Kowalska, Michał B. Czerwiński, Władysław Średniawa, Joanna Jędrzejewska-Szmek, Jakub M. Dzik, Daniel K. Wójcik

P6 – NEURONAL SIGNALING I

P6.1 **A link between glutamine metabolism and selective neuronal vulnerability in the hippocampus**
Paweł Bochomulski, Olga Krupska, Patrycja Klimczak, Wojciech Hilgier, Barbara Zabłocka, Małgorzata Beręsewicz

P6.2 **Loop G of the GABAA receptor orthosteric binding site is involved in the final stages of channel gating**
Marek Brodzki, Michał A. Michałowski, Michalina Gos, Jerzy W. Mozrzymas

P6.3 **Matrix metalloproteinase 3 critically affects postsynaptic long-term potentiation at GABAergic synapses in the mouse hippocampal CA1 region**
Patrycja Brzdąk, Katarzyna Lebida, Jerzy W. Mozrzymas

P6.4 **Lamotrigine affects theta oscillations in the hippocampus of rats**
Bartosz Caban, Joanna E. Sowa, Marcin Siwiec, Renata Bocian, Tomasz Kowalczyk, Paulina Kaźmierska-Grębowska

P6.5 **Characteristics of rat ventral tegmental area GABAergic-like neuronal responses to an aversive stimulus**
Gniewosz Drwięga, Magdalena Walczak, Kamil Pradel, Gabriela Izowit, Wojciech B. Solecki, Tomasz Błasiak

P6.6 **The response of main subclasses of GABAergic interneurons to plasticity induction and aging: Changes in mRNA levels**
Aleksandra Różycka, Renata Zakrzewska, Monika Liguz-Lęcznar

P6.7 **Decoding the spontaneous in vivo Ca2+ oscillations in zebrafish brain neurons**
Rishikesh Kumar Gupta, Iga Wasilewska, Oksana Palchevska, Jacek Kuźnicki

P6.8 **Expression of cannabinoid receptor type-1 (CB1) in different subpopulations of kisspeptin neurons in the mouse brain**
Imre Kalló, Mohanraj Mahendravarman, Krisztina Nagy, Kamil Ziarniak, Joanna H. Śliwowska, Tamás Wilheim

P6.9 **Increase of intrinsic excitability of neocortical somatostatin-expressing cells in layer IV of the mouse primary somatosensory cortex as a result of associative learning**
Dominik Kanigowski, Joanna Urban-Ciećko

P6.10 **Neurogenesis and behavioral strategies of in ICER overexpressing rats**
Agata Klejman, Artur Janusz, Anna Kiryk-Jaśkiewicz, Adam Gorlewicz, Katarzyna Biegańska, Paulina Jedynak, Leszek Kaczmarek, Witold Konopka

P6.11 **Posterior hypothalamic "timing cell" activity and kainite-induced local theta rhythm in both in vitro and in vivo preparations**
Tomasz Kowalczyk, Agata Staszelis, Paulina Kaźmierska-Grębowska, Joanna E. Sowa, Marcin Siwiec, Bartosz Caban

P6.12 **GABAergic plasticity in hippocampus depends on the activity of matrix metalloprotease-3**
Anna Lech, Anna Buszka, Jerzy W. Mozrzymas, Grzegorz Wiera

P6.13 **The transcription factor TCF7L2 governs postmitotic embryonic development of the thalamus and adult intrinsic excitability of thalamic neurons**
Marcin A. Lipiec, Kamil Koziński, Tomasz Zajkowski, Michał Dąbrowski, Chaitali Chakraborty, Joanna Urban-Ciećko, Angel Toval, José Luis Ferran, Andrzej Nagalski, Marta B. Wiśniewska

POSTER SESSION II

P7 – LEARNING AND BEHAVIOR II

P7.1 **Time increases the odds of repeating a previous choice**
Jan Rodriguez Parkitna, Łukasz Szumiec, Judyta Jabłońska

P7.2 **Inhibition and activation of the central amygdala circuits involved in social interaction suppresses motivation for sucrose reward**
Karolina Rojek-Sito, Ewelina Knapska

P7.3 **Investigation into the effects of chronic antidepressant and acute ketamine treatment on ultrasonic vocalisations in Wistar-Kyoto and Sprague-Dawley rats**
Fionn Dunphy-Doherty, Jack Prenderville, Massimiliano Bianchi, Ewa Sokolowska

P7.4 **Mice with a deletion of the mammal-specific microRNA 379-410 cluster display altered ultrasonic communication**
Ayse Özge Sungur, Martin Lackinger, Lea Stemmler, Robert Grosse, Rainer Schwarting, Gerhard Schratt, Markus Wöhr

P7.5 **Adult neurogenesis in opossums and hippocampal-dependent learning**
Beata Tepper, Agata Aniszewska, Katarzyna Bartkowska, Lilianna Grochocka, Krzysztof Turlejski, Ruzanna Djavadian

P7.6 **Fear conditioning affects reaction to ultrasonic signals in SHR and Wistar rats**
Agnieszka Wardak, Rafał Polowy, Aneta Grymanowska, Robert K. Filipkowski, Krzysztof H. Olszyński

P7.7 **Motor information increases visual awareness ratings: A TMS-MEP study**
Justyna Hobot, Marcin Koculak, Kristian Sandberg, Michał Wierzchoń

P7.8 **Intra ventral tegmental area noradrenergic receptors signalling regulates cue-induced fear memory retrieval**
Katarzyna Zajda, Gniewosz Drwięga, Paulina Wira, Klaudia Szklarczyk , Wojciech B. Solecki

P7.9 **5-HT7 receptors on GABAergic neurons modulate the inhibitory tone to principal cells in the mouse basal amygdala**
Izabela Ciurej, Magdalena Kusek, Marcin Siwiec, Joanna E. Sowa, Wiktor Bilecki, Marzena Maćkowiak, Krzysztof Tokarski

P7.10 **Up-regulation of PI3K-Akt-mTor signaling pathway in neurons affects cognitive functions and social interactions in a mouse model**
Natalia Chwin, Anna Kiryk, Łukasz Bijoch, Adam Hamed, Witold Konopka

P8 – DISEASE MODELS II

P8.1 **Methyl-CpG binding domain 3 promoter activity in a rat model of seizure evoked by intraperitional injection of pentylenetetrazol (PTZ)**
Karolina Nizińska, Aleksandra Stępniak, Katarzyna Łukasiuk

P8.2 **Hyperoside isolated from Impatiens glandulifera Royle alleviates depressive and anxiety-like responses in a mouse model of posttraumatic stress disorder**
Jolanta Orzelska-Górka, Katarzyna Szewczyk, Ewa Kędzierska, Ewelina Głowacka, Marta Kruk-Słomka, Grażyna Biała

P8.3 **Mechanism of MMP-9-1562C/T single nucleotide polymorphism-dependent regulation of MMP-9 (Matrix Metaloproteinase-9) expression in human neurons**
Sylwia Pabian-Jewuła, Magdalena Ambrożekk-Latecka, Aneta Brągiel-Pieczonka, Marta Grabiec, Marcin Rylski

P8.4 **Location of HuR protein in a model of photothrombotic ischemic stroke in rat - Method Development**
Katarzyna Pawletko, Halina Jędrzejowska-Szypułka

P8.5 **Pups' altered ultrasonic vocalisation in the poly I: C rat model of autism spectrum disorder: results from the mother isolation test**
Diana Piotrowska, Justyna Sopel, Agnieszka Potasiewicz, Piotr Popik, Agnieszka Nikiforuk

P8.6 **NMDA receptor modulation of the pedunculopontine tegmental nucleus decreases the number of tyrosine hydroxylase positive cells in the ventral tegmental area and substantia nigra pars compacta in rats**
Karolina Plucińska, Aleksandra Piwka, Kacper Ptaszek, Magdalena Podlacha, Jolanta Orzeł-Gryglewska, Grażyna Jerzemowska

P8.7 **Let genes talk: transcriptomic changes in the PFC induced by L-DOPA in hemiparkinsonian rats**
Anna Radlicka, Kinga Kamińska, Marcin Piechota, Michał Korostyński, Elżbieta Lorenc-Koci, Joanna Pera, Jan Rodriguez Parkitna

P8.8 **EBI2 knock-out mice show greater loss of brain lipids followed by earlier attempts at remyelination in the cuprizone model of demyelination**
Joanna Klimaszewska-Łata, Derya R. Shimshek, Andrzej Szutowicz, Aleksandra Rutkowska

P8.9 **The potent role of catecholaminergic innervation in locomotor recovery induced by intraspinal grafting of embryonic brainstem tissue in adult paraplegic rats**
Anna Bejrowska, Krzysztof Miazga, Henryk Majczyński, Małgorzata Zawadzka, Urszula Sławińska

P8.10 **Impact of ketogenic diet on gait in Purkinje cell specific transgenic mouse model of tuberous sclerosis complex**
Anna Sługocka, Marta Grabowska, Jarosław J. Barski

P8.11 **Impact of environmental enrichment on anxiety and learning in the rat model of epilepsy induced by electrical stimulation of the amygdala**
Aleksandra Stępniak, Karolina Nizińska, Kinga Szydłowska, Katarzyna Łukasiuk

P8.12 **Cerebral administration of alpha-synuclein modulates inflammatory reaction in the nigro-striatal system: A comparison of males and females**
Anna Sznejder-Pachołek, Ilona Joniec-Maciejak, Adriana Wawer, Ewa Wojnar, Dagmara Mirowska-Guzel

P8.13 **HFO under ketamine-xylazine anesthesia are coupled to large current sources and nasal respiration: A proposed mechanism for the generation of HFO after NMDAR blockade**
Władysław Średniawa, Jacek J. Wróbel, Miles Whittington, Daniel K. Wójcik, Mark J. Hunt

P8.14 **Prenatal stress-induced sex differences in the incidence and the course of experimental autoimmune encephalomyelitis (EAE) in adult rats: offspring of mothers with different sensitivity to EAE**
Svitlana Utevska, Valentina Geyko, Olha Berchenko

P8.15 **Neuroprotective properties of cystamine in MPTP-induced murine model of Parkinson's disease**
Adriana Wawer, Ilona Joniec-Maciejak, Anna Sznejder-Pachołek, Ewa Wojnar, Dagmara Mirowska-Guzel

P8.16 **The effect of a sphingosine-1-phosphate receptors modulator on the transcriptional profile of genes linked to glutamate homeostasis in the sporadic and genetic animal models of Alzheimer's disease**
Przemysław Wencel, Grzegorz Sulkowski, Beata Dąbrowska-Bouta , Robert P. Strosznajder, Lidia Strużyńska

P8.17 **Fingolimod (FTY720 – modulator of sphingosine-1-phosphate receptors) alters genes expression of selected NAD+-dependent enzymes in an animal model of Alzheimer's disease**
Przemysław Wencel, Henryk Jęśko, Robert P. Strosznajder

P8.18 **Nasal respiration is critical for ketamine-induced high frequency oscillations and hyperactivity**
Jacek J. Wróbel, Władysław Średniawa, Daniel K. Wójcik, Mark J. Hunt

P8.19 **Maternal immune activation during pregnancy alters the expression of mitochondrial dynamics markers in the brain of rat offspring**
Aleksandra Zawadzka, Magdalena Cieślik, Magdalena Gewartowska, Małgorzata Frontczak-Baniewicz, Agata Adamczyk

P9 – STRESS RESEARCH

P9.1 **Role of α2-adrenergic receptors in ventral tegmental area in the phasic dopamine release into basolateral amygdala in stressed and non-stressed rats**
Joanna Bernacka, Michał Kiełbiński, Wojciech B. Solecki

P9.2 **Maternal separation stress alters excitability and stress induced c-fos expression in ventral tegmental area dopaminergic neurons of adult female rats**
Anna Guguła, Jadwiga Spyrka, Katarzyna Cizio, Grzegorz Hess, Anna Błasiak

P9.3 **Does an extremely low frequency electromagnetic field (50 Hz) have a permanent effect on the stress- related behaviour in rats?**
Angelika Klimek, Agnieszka Siejka, Hanna Kletkiewicz, Justyna Maliszewska, Joanna Wyszkowska, Maria Stankiewicz, Justyna Rogalska

P9.4 **The role of cortisol and kisspeptin in suppression of the GnRH/LH secretion in follicular-phase ewes subjected to prolonged stress**
Magdalena Ciechanowska, Magdalena Łapot, Marek Kowalczyk, Marek Brytan, Franciszek Przekop

P9.5 **The effect of low frequency electromagnetic field (50 Hz) on noradrenaline levels in the hypothalamus in rats**
Agnieszka Siejka, Angelika Klimek, Hanna Kletkiewicz, Justyna Maliszewska, Joanna Wyszkowska, Maria Stankiewicz, Marek Wieczorek, Justyna Rogalska

P10 – NEURONAL SIGNALING II

P10.1 **Implication of GABA-ergic system on GnRH and GnRHR biosynthesis and LH secretion in the hypothalamic-pituitary unit of follicular-phase ewes**
Magdalena Łapot, Marek Brytan, Marek Kowalczyk, Franciszek Przekop, Magdalena Ciechanowska

P10.2 **Somatostatin receptors on parvalbumin interneurons in mouse somatosensory cortex**
Agnieszka Łukomska, Grzegorz Dobrzański, Monika Liguz-Lęcznar, Małgorzata Kossut

P10.3 **Investigation of Ca^{2+} homeostasis and behavioral changes in transgenic mice overexpressing key store-operated calcium entry proteins**
Filip Maciąg, Łukasz Majewski, Paweł M. Boguszewski, Jacek Kuźnicki

P10.4 **Molecular mechanisms of proton modulation in the $GABA_A$ receptor as compared to low pH activation scheme in the GLIC ion channel**
Michał A. Michałowski, Jerzy W. Mozrzymas

P10.5 **Superior colliculi control activity of the rostromedial tegmental nucleus in a lateralized manner – an optogenetic study in the rat**
Kamil Pradel, Tomasz Błasiak

P10.6 **Neuromodulatory control of long-term synaptic plasticity in CA1 pyramidal neurons**
Joanna Jędrzejewska-Szmek, Ziemowit Sławiński, Daniel K. Wójcik

P10.7 **Creation of the neocortical layers and their connections in the brain of Monodelphis domestica**
Paulina Sobolewska

P10.8 **Electrophysiological effects of CX3CL1 on rat amygdala neurons**
Joanna E. Sowa, Anna Solarz, Agnieszka Chocyk, Grzegorz Hess, Krzysztof Tokarski

P10.9 **NMDA-induced posterior hypothalamic theta rhythm recorded in vitro**
Agata Staszelis, Bartosz Caban, Paulina Kaźmierska-Grębowska, Joanna E. Sowa, Marcin Siwiec, Tomasz Kowalczyk

P10.10 **$GABA_A$ β2 subunit loop C shapes binding and gating properties of receptor activation**
Katarzyna Terejko, Przemysław T. Kaczor, Michał A. Adamowski, Agnieszka Dąbrowska, Jerzy W. Mozrzymas

P10.11 **Electrophysiological characterization of the rat nucleus incertus neurons in relation to hippocampal theta oscillations**
Aleksandra Trenk, Magdalena Walczak, Tomasz Błasiak

P10.12 **Stim2 role in response to oxidative stress**
Iga Wasilewska, Oksana Palchevska, Rishikesh Kumar Gupta, Jacek Kuźnicki

P10.13 **Screen for kinesins critical in mTOR-dependent neuronal development**
Jan Węsławski, Joanna Lipka, Jacek Jaworski

P11 – NEURONAL METABOLISM

P11.1 **Acute normobaric hypoxia lowers executive functions despite an increase in BDNF levels**
Maciej Chroboczek

P11.2 **Impact of ketogenic diet on Enterococcus faecalis in the gut flora of Long Evans and Wistar rat strains**
Mateusz Grabowski, Konstancja Jabłońska

P11.3 **The influence of ketogenic diets on the amount of Enterococcus faecalis and Escherichia coli in faeces of the 129SV mice**
Konstancja Jabłońska, Mateusz Grabowski

P11.4 **The ability of BDNF to decrease caspase-3 level depends on body temperature under anoxic conditions**
Hanna Kletkiewicz, Agnieszka Siejka, Angelika Klimek, Justyna Rogalska

P11.5 **Effect of aerobic and resistance interval exercises on peripheral concentration of neuroprotective proteins and human cognitive abilities**
Sylwester Kujach, Robert Olek, Maciej Chroboczek, Tomasz Figuła, Radosław Laskowski

P11.6 **Anxiolytic effect of physical activity and cerebral accumulation of saturated fatty acids**
Arkadiusz Liśkiewicz, Marta Przybyła, Anna Wojakowska, Łukasz Marczak, Katarzyna Bogus, Marta Nowacka-Chmielewska, Daniela Liśkiewicz, Andrzej Małecki, Jarosław J. Barski, Joanna Lewin-Kowalik, Michał Toborek

P11.7 **Ketogenic diets based on fat of either animal or plant origin have different effects on the abundance of autophagic vesicles in mouse brain**
Daniela Liśkiewicz, Arkadiusz Liśkiewicz , Marta Nowacka-Chmielewska, Konstancja Jabłońska, Mateusz Grabowski, Sebastian Student, Anna Sługocka, Marta Przybyła, Jarosław J. Barski, Andrzej Małecki

P11.8 **Effects of diet-induced obesity and diabetes type 2 on neuropeptide Y-immunoreactive neurons in the hypothalamus of male and female rats**
Julia Matuszewska

P11.9 **Anxiety-like behavior of laboratory animals on a ketogenic diet**
Marta Przybyła, Daniela Liśkiewicz, Marta Nowacka-Chmielewska, Arkadiusz Liśkiewicz, Aniela Grajoszek, Mateusz Grabowski, Konstancja Jabłońska, Jarosław J. Barski

P11.10 **Effects of ovariectomy and sex hormone replacement on the number of neurokinin B-immunoreactive neurons in the arcuate nucleus of the hypothalamus of obese and diabetic female rats**
Kamil Ziarniak, Paweł A. Kołodziejski, Ewa Pruszyńska-Oszmałek, Maciej Sassek, Monika Dudek, Joanna H. Śliwowska

P11.11 **The influence of various compositions of the ketogenic diet on the behavior of selected mouse strains**
Aniela Grajoszek, Konstancja Jabłońska, Mateusz Grabowski, Jarosław J. Barski

PLENARY LECTURES

PL1. NOVEL MECHANISMS OF NEUROGENESIS AND NEURAL REPAIR

Magdalena Götz

Institute for Stem Cell Research, Helmholtz Center Munich and Biomedical Center, University of Munich, Munich, Germany

We study the mechanisms of neurogenesis in order to implement them for neuronal repair. I will present unpublished work about the molecular function of Trnp1, a novel nuclear protein, with key roles in promoting neural stem cell self-renewal and neurogenesis. Trnp1 shows unprecedented functions in regulating several nuclear processes by its N-terminal intrinsically disordered region, which is highly conserved in mammals. I will then show that Trnp1 is also critical for direct neuronal reprogramming and provide an update on the recent breakthrough in direct glia-to-neuron conversion after brain injury. I will then move on to discuss the integration of replaced neurons into the circuitry of the murine cerebral cortex – that normally does not integrate new neurons at adult stages – and present unpublished data about the mechanisms regulating this integration. Taken together, our knowledge about basic mechanisms of neurogenesis allows us to make great strides towards neuronal repair.

PL2. GABAERGIC NEURONES – THE CELLULAR SUBSTRATE FOR LOCAL AND LONG-RANGE SYNCHRONY

Hannah Monyer

Department of Clinical Neurobiology, Medical Faculty of Heidelberg University and German Cancer Research Center (DKFZ), Heidelberg, Germany

Over the past decade we used genetic manipulations to study the contribution of GABAergic interneurones for rhythmic synchronous activity. We focused on the hippocampus on the medial entorhinal cortex, two brain structures that are crucially involved in spatial coding and spatial memory. Genetic manipulations included ablations of glutamate receptors or electrical coupling in GABAergic interneurones in the whole forebrain, or locally in the hippocampal-entorhinal formation. Our studies underline the functional role of local GABAergic interneurones for spatial or temporal coding in the hippocampus. The genetic manipulations were always associated with distinct spatial memory deficits. These results will be summarized and discussed in the context of current models of memory formation and storage. In addition, I will present data demonstrating the presence of long-range GABAergic cells that connect the hippocampus and entorhinal cortex reciprocally. Also these data will be discussed in a larger context, since there is good reason to believe that long-range GABAergic neurones are more abundant in the forebrain as previously thought. Most recent studies in the lab focus on long-range GABA that connect several cortical areas involved in pain perception. By virtue of their connectivity – the target cells are most often local interneurones – this class of cells is ideally suited to synchronize brain regions over long distance.

PL3. MULTIPLE SCLEROSIS: FORMATION OF A BRAIN AUTOIMMUNE DISEASE

Hartmut Wekerle

Max Planck Institute of Neurobiology, Martinsried, Germany

Recent clinical and experimental studies indicate that multiple sclerosis (MS) develops as consequence of a failed interplay between genetic and environmental factors. An ever-growing number of risk genes have been recognised that support an autoimmune response against the body's own brain matter. Together, these increase susceptibility to MS without actually triggering the disease. Recent experimental observations indicate that the actual trigger of the autoimmune attack is provided by an interaction of brain-specific immune cells with microbial organisms. Unexpectedly, these microbes are not necessarily responsible for infections, but are components of the healthy commensal gut flora – the intestinal microbiota. This concept opens the way for new therapeutic approaches involving modulation of the microbiota by dietary or antibiotic regimens.

PL4. PLASTICITY OF THE PRIMATE VISUAL PATHWAY FOLLOWING LESIONS IN EARLY AND MATURE LIFE

Marcello Rosa

Biomedicine Discovery Institute and Department of Physiology, Monash University, Melbourne, Australia

In primates, visual function is dominated by the pathway that transmits visual information from the retina, via the lateral geniculate nucleus (LGN), to the primary visual cortex (V1). Although lesions of V1 lead to blindness, it is well documented that residual visual function can be retained within scotomas caused by V1 lesions, including (largely subconscious) abilities to locate some types of stimuli, and even to coarsely evaluate their characteristics ("blindsight"). These observations indicate that other thalamic projections can convey retinal inputs directly to the extrastriate cortex, bypassing V1. The exact characteristics of blindsight depend

markedly on the age at which the lesion occurs. Patients and monkeys who sustained lesions early in life often show a greater range of abilities than those who had lesions in adulthood, including, in many cases, conscious perception. My laboratory has been investigating the types of physiological changes in subcortical and cortical areas which mediate such outcomes. For this purpose, we have developed a V1 lesion model based on the marmoset monkey, a small new world primate in which the anatomy and physiology of the visual pathways has been well characterised, and has accelerated development in comparison with macaque monkeys. In this talk, I will briefly review the characteristics of the marmoset as an advantageous animal model for studies of primate vision, including plasticity, describe recent findings on the physiological consequences of V1 lesions at different ages, and briefly report on current lines of work aimed at understanding the full circuitry of the marmoset visual cortex using a neuroinformatics approach.

PL5. PRECLINICAL DEVELOPMENT OF TREATMENTS FOR TAUOPATHIES: TRANSLATION TO THE CLINIC AND BACK

Gernot Riedel

University of Aberdeen, Institute of Medical Sciences, Aberdeen, UK

Given the urgent need for a disease modifying treatment of Alzheimer's disease (AD), there is increasing interest in tau-based therapeutics. In a comparative study, methylthioninium chloride (MTC) and leucomethylthioninium salts (LMTX®) (5-75 mg/kg; oral administration for 3-8 weeks) were assessed preclinically in two novel transgenic tau mouse lines (Line 1, Line 66). Behavioural and histopathological proxies were evaluated. Both MTC and LMTX® dose-dependently rescued the learning impairment and restored behavioural flexibility in a spatial problem-solving water maze task in Line1 and corrected motor-learning in Line 66. Simultaneously, both drugs reduced the number of tau-reactive neurons, particularly in the hippocampus and entorhinal cortex in Line 1 and had more widespread effects in Line 66. The data establish that diaminophenothiazine compounds like MTC can reverse both spatial and motor learning deficits and reduce the underlying tau pathology and therefore offer potential for the treatment of tauopathies.

In the clinic, symptomatic treatments with cholinesterase inhibitors and/or memantine are relatively ineffective and the need for new treatments targeting the underlying pathology of AD is generally recognised. In most of the failed disease-modifying trials conducted over the last 16 years, patients have been allowed to continue taking symptomatic treatments at stable doses, under the assumption that they do not impair efficacy because the modes of action are different. In recently completed Phase 3 trials testing the tau aggregation inhibitor leuco-methylthioninium bis (hydromethanesulfonate) (LMTM), we found highly significant differences in treatment response according to whether patients were taking LMTM as monotherapy (benefit) or as add-on to symptomatic treatments (no effect).

A large body of preclinical research has then been undertaken in wild-type mice and in our tau transgenic mouse model (Line 1) expressing the core tau unit of the AD paired helical filament with the aim of understanding the mechanisms responsible for the reduced efficacy of LMTM as an add-on to symptomatic treatments. A range of experimental paradigms were used to measure the effects of chronic pretreatment with the cholinesterase inhibitor rivastigmine given for 2-5 weeks prior to adding LMTM treatment for a further 2-6 weeks. In tau transgenic mice, LMTM given alone was found to increase hippocampal acetylcholine (ACh) levels, glutamate release from synaptosomal preparations, synaptophysin levels in multiple brain regions, mitochondrial complex IV activity, reduce tau pathology, restore choline acetyl transferase (ChAT) immunoreactivity in basal forebrain, and reverse deficits in spatial learning. Chronic pretreatment with rivastigmine was found to reduce or eliminate almost all LMTM treatment effects, apart from reduction in tau aggregation pathology and restoration of ChAT immunoreactivity in the basal forebrain. LMTM effects on hippocampal ACh and levels of synaptophysin were also reversed in wild-type mice.

Collectively, targeting tangles consisting of MAPT protein tau is a viable strategy in preclinical models and was forward translated to AD patients receiving monotherapy. In the clinic, however, prior symptomatic treatment with a cholinesterase inhibitor prohibited the efficacy of LMTM. Back translation to our tau mouse model reproduced this negative interaction and revealed a mechanistic action across different transmitter systems and at multiple compartmental levels of neural function.

PL6. MECHANOBIOLOGY OF THE BRAIN IN HEALTH AND DISEASE

Graham K. Sheridan

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Basic research into neurodegenerative disorders, like Alzheimer's disease, is heavily focused on understanding genetic susceptibility and biochemical triggers of pathology, as well as disturbances to the intrinsic electrophysiological properties of affected neurons. Often overlooked is the role of mechanics, particularly mechanical

properties and mechano-sensitivity/-responsiveness of neurons and glia. Recent evidence confirms that mechanical signals regulate CNS development and pathophysiology. In this talk, I will discuss the role of mechanics in both physiological and pathophysiological brain ageing. A defining pathophysiological hallmark of Alzheimer's disease is the amyloid plaque; an extracellular deposit of aggregated fibrillar $A\beta_{1-42}$ peptides. Amyloid plaques are hard, brittle structures scattered throughout the hippocampus and cerebral cortex and are thought to cause hyperphosphorylation of tau, neurofibrillary tangles, and progressive neurodegeneration. Glia are highly mechanosensitive cells and can sense the mismatch between the normally soft mechanical environment of the brain and very stiff amyloid plaques via mechanosensing ion channels. Both ageing and peripheral infection augment amyloid plaque-induced upregulation of mechanoresponsive ion channels in astrocytes. Further research is required to investigate whether modulating mechanically-gated channel opening will protect or exacerbate the disease state, and most importantly, if they are novel drug targets for age-related dementia.

SYMPOSIA LECTURES

S1.1. EMISSION OF 50-KHZ ULTRASONIC VOCALIZATIONS IN DOPAMINE-DENERVATED RATS TREATED WITH AMPHETAMINE: RELEVANCE TO NEUROCIRCUITRIES INVOLVED IN DRUG-MEDIATED REWARD

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Rats emit 50-kHz ultrasonic vocalizations (USVs) in response to pleasurable stimuli, and USVs are increasingly being used to investigate the affective properties of drugs. Dopamine in the shell of the nucleus accumbens is instrumental in the emission of 50-kHz USVs, but little is known about how calling behavior is modulated by other brain regions that receive dopaminergic innervation. To clarify this issue, we evaluated calling behavior stimulated by repeated amphetamine administration in rats subjected to either bilateral or unilateral dopaminergic denervation with 6-OHDA in the medial prefrontal cortex (mPFC), dorsolateral striatum (DLS), or medial forebrain bundle (MFB). Dopaminergic denervation in the PFC, DLS, or MFB only partially attenuated the development of 50-kHz USVs sensitization during repeated treatment with amphetamine. However, rats bearing a dopaminergic denervation in the mPFC emitted a low number of conditioned USVs upon re-exposure to the amphetamine-paired environment. Sensitization in ultrasonic calling and emission of 50-kHz USVs conditioned to an environment previously paired with drug administration have recently emerged as behavioral correlates of the motivational properties of drugs of abuse. Accordingly, the present results may provide new insight into the neurocircuitries involved in reward and motivation mediated by addictive drugs.

S1.2. SOCIAL INTERACTION AND ACOUSTIC COMMUNICATION IN ADULT MICE: MARKERS FOR HEALTHY AND PATHOLOGICAL BEHAVIORS

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Adapted social behavior allows both individual and collective well-being. At the individual level, it is a hallmark of health. Indeed, virtually all mental health disorders are associated with social deficits. We are interested in understanding the behavioral, neural, and neurochemical bases of social cognition and communication using mouse models. Here, we will review our recent data showing the crucial role of the prefrontal cortex in the organisation of adapted social interaction, the interplay between the cholinergic and the noradrenergic systems for the balance between affiliative interaction, dominance, and control of aggressiveness, and we will discuss the putative role of ultrasonic communication in social interactions in adult animals. We will see the role played by the environment of life and by the context in which interactions take place in healthy individuals and in pathological situations. Together, the data presented will offer a novel focus on the social brain – and social life – of rodents and provide some practical recommendations for future experiments.

S1.3. BEHAVIORAL, ULTRASONIC, AND CARDIOVASCULAR RESPONSES OF MALE WISTAR RATS IN DIFFERENT SOCIAL AND EMOTIONAL CONTEXTS AFTER ULTRASONIC PLAYBACK

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Rats are social animals that use ultrasonic vocalizations (USV) to communicate. USV are usually divided into 50 kHz calls which accompany appetitive states, and 22 kHz vocalizations which are usually associated with aversive states. Both kinds of states are known to affect animals' heart rate (HR). Also, the polyvagal theory claims that both cardiovascular parameters and USV emission is affected by the autonomous system, as

they share a common signaling pathway. The aim of the study was to evaluate the changes in behavior, HR, and USV emission after playback of ultrasounds. Male Wistar rats were housed in pairs or separately for 4 weeks, and some of the animals underwent fear conditioning. Animals were implanted with DSI telemetry transmitters for acquisition of cardiovascular parameters. After recovery, rats were subjected to ultrasounds playback consisting of initial 10 min of static silence and five sets of 10 s sounds (50 or 22 kHz), either natural, collected from animals, or artificial tones, separated by 5 min silence intervals. Video, audio, and cardiovascular parameters were collected. Surprisingly, presentation of both 50 and 22 kHz sounds induced approach behavior. Both single- and pair-housed animals responded with a larger number of USV to both natural and artificial 50 kHz sounds playback rather than to 22 kHz sounds. The emitted USV were, almost exclusively, within the 50 kHz range. Animal HR levels decreased gradually during the experimental session. Single-housed animals had, in general, higher HR than paired rats. There was an impact of every kind of ultrasonic presentation on HR levels; in general, 50 kHz ultrasonic playback caused a sudden increase in HR, whereas 22 kHz presentations evoked a HR drop. Surprisingly, USV and artificial tones had similar effects on HR and USV responses. Social context did not appear to alter rats' USV emission. The results following fear conditioning are being analyzed. Also, in a separate set of experiments, rats ultrasonic responses were analyzed following presentation of a defined number of pre-recorded USV.

S1.4. NEUROCHEMICAL CORRELATES OF 50-KHZ ULTRASONIC VOCALIZATIONS IN CONTEXT INDUCED RESPONSE, REWARD PROCESSING, AND APPETITIVE SOCIAL INTERACTIONS

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A tool that most effectively determines the emotional states of rats is the registration and analysis of ultrasonic vocalizations (USVs). 50-kHz USVs are a form of expression of positive emotions. USV measurement allows both identifying individual differences in processing information about the reward as well as reflecting, to a large extent, the level of individual motivation. We hypothesized that 50-kHz USV emission could have separate neurochemical backgrounds in different behavioral paradigms, as well as, some common neurochemical mechanisms reflected in examined neurotransmitters correlations. For example, re-exposure to the context of morphine administration is associated with elevated serotonin concentrations in the amygdala, hippocampus, and medial prefrontal cortex (mPFC) and increased Glu/Gln ratio in the nucleus accumbens (Nacc). Machine learning based analysis indicates a strong correlation between serotonergic and glutamatergic systems in context-induced conditioned response. In the case of social interaction paradigm, several neurochemical changes were detected. Depending on the duration of social interaction, neurotransmission pathways are activated in the cascade fashion. Thus, glutamatergic neurotransmission in amygdala, ventral tegmental area (VTA), Nacc, and hippocampus, and action in the serotonergic system in mPFC, Nacc, caudate, and putamen, dopaminergic neurotransmission in mPFC and hippocampus, and noradrenergic neurotransmission in the striatum, are activated under the influence of the elapsed time of social interaction.

S2.1. GUIDED TOUR OF PROTEINS INTO MITOCHONDRIA

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Mitochondria are multifunctional organelles, primarily involved in the fundamental biological process of respiration. The efficient functioning of mitochondria depends on the proper transport, sorting, and assembly of mitochondrial proteins that originate either from nuclear or mitochondrial genomes. Both nuclear and mitochondrial gene defects that result in pathological variants of proteins have been implicated in a variety of mitochondrial diseases. The nuclear-encoded proteins make up the large majority of proteins involved in the formation of mitochondria, including the respiratory chain complexes. The ubiquitin proteasome system (UPS) in the cytosol is involved in degradation of cellular proteins and maintaining protein homeostasis. By multiple lines of evidence, we have demonstrated the contribution of the UPS to mitochondrial protein quality control. The UPS degrades a portion of mitochondrial proteins, including mislocalized proteins, in both yeast and mammalian systems. Furthermore, mislocalization of mitochondrial proteins increases the ability of the proteasome to degrade cellular proteins. Thus, the UPS constitutes an important factor that affects the mitochondrial protein import, influences the mitochondrial proteome, and links the mitochondrial status with regulation of cellular protein homeostasis. Interestingly, pathologic variants of mitochondrial proteins can be mistargeted and fully degraded by the proteasome before they reach their final destination inside mitochondria. Inhibition of proteasomal degradation by com-

monly used proteasome inhibitors results in rescue of proteins and their import into the mitochondria. Thus, UPS inhibition can provide a benefit to malfunctioning mitochondria and cells. We propose that targeting the UPS should be considered as a therapeutic strategy for mitochondrial diseases.

S2.2. MITOCHONDRIAL INTEGRITY AND FUNCTION IN MODEL SYSTEMS OF CNS DISEASES

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Mitochondria are key regulators of energy metabolism, redox balance, calcium homeostasis, and programmed cell death. In the past, we characterized mitochondria acting as targets of both caspase-dependent and caspase- independent death signalling triggered by increased oxidative stress and as executioners of programmed death signalling in neurons. For example, we identified mitochondrial damage in caspase-independent neuronal death after cerebral ischemia *in vivo*, and in oxidative cell death, i.e., ferroptosis *in vitro*. Protective intervention against oxidative damage further confirmed the conclusion that mitochondria represent the "point of no return" in caspase-independent paradigms of programmed cell death. Further, we found more recently that mitochondri al-targeted alpha-synuclein caused severe mitochondrial toxicity and caspase-dependent cell death in human dopaminergic neurons, a model system relevant to Parkinson's disease. In different model systems of neuronal death, neuroprotective interference with mitochondrial pathways of programmed cell death was frequently attributed to metabolic switches, i.e., reduced mitochondrial respiration and increased glycolytic activity. Accordingly, targeting metabolic switches may serve as a general strategy for mitochondrial protection and, thereby, neuroprotection, but may also affect mechanisms of neuroinflammation involving activation of microglia. The understanding of the underlying mechanism of such metabolic protection may reveal novel therapeutic targets in neural diseases featuring mitochondrial impairments and neuroinflammation.

S2.3. BRAIN ENERGY METABOLISM IN NEURODEGENERATION OF DOPAMINERGIC NEURONS IN ANIMAL MODEL OF EARLY PARKINSON'S DISEASE: ROLE OF ASTROCYTES

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Glial pathology and energy metabolism changes in the brain precede symptoms of Parkinson's disease (PD) and multiple other neurodegenerative diseases. Astrocytes govern and regulate a large part of the energy metabolism in the brain. Prolonged impairment of astrocytic functions could increase the vulnerability of dopaminergic neurons in the substantia nigra (SN). In this model, 40-50% of dopaminergic neurons were selectively killed, causing transient locomotor disability compensated with time. We also induced death of astrocytes in the SN, simultaneously activating microglia but sparing the dopaminergic neurons. The astrocytes replenished after toxin withdrawal. We studied multiple markers of energy metabolism and mitochondrial oxidative phosphorylation (OxPhos) complex and supercomplex functioning during the early stages of neurodegeneration and compensation in the SN and striatum (STR). Death of astrocytes diminished the capability of the dopaminergic system to compensate for the degeneration of neurons. It caused a local energy deprivation, a shift in the usage of energy substrates, via increased glycogenolysis and glycolysis markers, ketone bodies availability, and fatty acid transport in remaining glial cells. Increased neuronal expression of CPT1c and astrocytic expression of CPT1a suggest adaptation in fatty acid use. On the other hand, lesion of dopaminergic neurons influenced OxPhos system and enhanced its functioning. Microglia activation also plays an important role in the processes of degeneration, compensation, and energy metabolism regulation. Modulation of its activation phenotypes might be beneficial towards the indicated processes. Astrocyte and microglia energetic influence is one of the factors in the neuronal compensatory mechanisms of dopaminergic system and might have a leading role in presymptomatic PD stages.

S2.4. THE ASSOCIATION BETWEEN MATERNAL IMMUNE ACTIVATION AND MITOCHONDRIAL FAILURE IN ADULTHOOD: RELEVANCE TO NEURODEVELOPMENTAL DISORDERS

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Evidence suggests that maternal immune activation (MIA) during pregnancy is a risk factor for neurodevelopmental disturbances including autism spectrum disorders (ASDs). Animal models support this linkage and demonstrate that MIA in rodents leads to behavioral alterations in offspring that are characteristic of autism. However, the mechanism by which MIA causes long-term behavioral deficits is unknown.

Investigation of the links between maternal infection during pregnancy, mitochondrial dysfunction, and behavioral alterations in offspring. To induce MIA, pregnant Wistar rats were injected with lipopolysaccharide (LPS; 0.1mg/kg, intraperitoneally) on gestational day 9.5, a time point analogous to the first trimester of human gestation. Brains from adolescent offspring were evaluated for mitochondrial outcomes. Prenatal exposure to MIA led to anxiety and repetitive behavior. Adolescent offspring of MIA dams exhibited up-regulation of pro-inflammatory cytokines, oxidative stress, and disturbances in redox homoeostasis. Moreover, substantial mitochondrial abnormalities were observed. A significant decrease in mitochondrial membrane potential and changes in ATP production could be attributed to a downregulation of complex I and IV. Deregulated bioenergetics of mitochondria were accompanied by impaired mitochondrial dynamics, altered expression of fusion/fission machinery proteins including mitofusin 1 and 2 (Mfn1, Mfn2), Opa1, dynamin related protein-1 (Drp1), and fission protein 1 (Fis1). We also demonstrated lower expression of the genes coding for PGC1α and TFAM (*PPARGC1A* and *TFAM*, respectively) that are responsible for mitochondrial biogenesis.

MIA at early gestation leads to long-lasting effects on the mitochondrial bioenergetics, dynamics, and biogenesis in the offspring which can lead to synaptic dysfunction and behavioral abnormalities similar to ASD.

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S2.5. GASTROINTESTINAL (GI) TRACT-DERIVED BACTEROIDETES-FRAGILIS NEUROTOXINS AND INFLAMMATORY NEURODEGENERATION

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The Gram-negative facultative anaerobe *Bacteroides fragilis* (*B. fragilis*) constitutes an appreciable proportion of the human gastrointestinal (GI)-tract microbiome. As is typical of most Gram-negative bacilli, *B. fragilis* secretes an unusually complex mixture of neurotoxins including 2 extremely pro-inflammatory species: (i) a *B. fragilis*-associated lipopolysaccharide BF-LPS; and (ii) a *B. fragilis*-derived proteolytic enterotoxin known as fragilysin (*EC 3.4.24.74*). BF-LPS has recently been shown to be associated with the periphery of neuronal nuclei in sporadic Alzheimer's disease (AD) brain; and the extracellular zinc metalloprotease fragilysin (i) induces endogenous E-cadherin cleavage thereby disrupting cell-cell adhesion and the GI-tract-blood barrier (GTBB); and (ii) promotes the generation of the inflammatory transcription factor NF-kB (p50/p65 complex) in human neuronal-glial cells in primary culture. In turn, the NF-kB (p50/p65 complex) strongly induces the transcription of a small family of pro-inflammatory microRNAs (miRNAs) including miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a, and miRNA-155. These ultimately bind with the 3'-untranslated region (3'-UTR) of several target messenger RNAs (mRNAs) and thereby reduce their expression. Down-regulated mRNAs include those encoding complement factor-H (CFH), an SH3-proline-rich multi domain-scaffolding protein of the postsynaptic density (SHANK3), and the triggering receptor expressed in myeloid/microglial cells (TREM2), as is observed in sporadic AD brain. Hence, a LPS and an enterotoxic metalloprotease normally confined to the GI tract are capable of driving a disruption in the GI-tract-blood barrier and a NF-kB-miRNA-mediated deficiency in gene expression that contributes to alterations in synaptic-architecture and synaptic-deficits, amyloidogenesis, innate-immune defects, and progressive inflammatory signaling, all of which are characteristic of AD-type neurodegeneration. This paper will review the most recent research which supports the concept that bacterial components of the GI-tract microbiome such as BF-LPS and fragilysin can transverse normally protective biophysical barriers and contribute to AD-type changes. For the first-time, these results indicate that specific GI-tract microbiome-derived neurotoxins have a strong pathogenic potential in disrupting the GI-tract blood barrier and eliciting alterations in NF-kB-miRNA-directed gene expression that drive the AD process.

S3.1. LOCATION, LOCATION, LOCATION: THE ROLE OF BRAIN ATLASES AND SPATIAL INTEGRATION OF MULTIMODAL IMAGING DATA IN WHOLE BRAIN MAPPING PROJECTS

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Spatial integration of multimodal imaging data is a common denominator of all whole brain mapping projects. This process requires robust image registration pipelines, high- quality 3D brain atlases, as well as, scalable methods for quantitative image analysis. During the talk, I will discuss the computational chal-

lenges behind these components, exemplify ways of addressing them, and discuss requirements for setting up one's own computational pipeline. I will also demonstrate how these novel computational methods and approaches could provide a deeper understanding of the structure and function of the central nervous system, especially in the context of high-throughput and large-scale experiments. The talk will be complemented with examples of specific neuroscientific findings arising from whole brain mapping projects in rodents and primates, highlighting synergy between the computational and experimental aspects in these projects.

S3.2. ADDICTED BRAIN MAPPING AND IMAGING USING LIGHT-SHEET FLUORESCENCE MICROSCOPY

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Addiction is a disease that affects circuits and brain areas involved in reward, stress, and self-control. Continued substance abuse can lead to dependence that is associated with withdrawal symptoms when drug availability is ceased, and increased intake upon relapse. With many brain structures involved in the disease, the whole-brain analysis is a promising strategy for gaining a deeper insight into events leading to addiction development and its particular aspects, such as relapse. We used a mouse model of alcohol addiction to perform whole-brain analysis of the activity during addiction-like behaviors. Mice were trained to drink ethanol in "drinking in the dark" paradigm adapted to the IntelliCage system. Afterwards, the animals were deprived of alcohol and tested for relapse. Whole hemispheres of each mouse were subjected to optical clearing, c-Fos staining, light-sheet imaging, and analyzed for c-Fos expression to identify brain areas involved in relapse.

S3.3. BRAIN-WIDE MAPPING OF OXYTOCIN RECEPTOR NEURONS IN THE DEVELOPING POSTNATAL MOUSE BRAIN

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Oxytocin is a neuropeptide with functions including the development and expression of social behavior. Despite its significance, brain-wide spatial and temporal expression patterns of the oxytocin receptor (OTR), the main mediator of the oxytocin ligand, remain largely unknown. Here, we examined the expression patterns of oxytocin receptor-positive neurons at different developmental time points (P7, P14, P21, P28) and in adulthood (P56) using a transgenic reporter line (OTR-GFP). We used serial two-photon tomography for whole brain imaging at cellular resolution and a data processing pipeline that allows us to map fluorescently labeled cells throughout the entire brain with our newly developed postnatal 3D atlases. We found temporally different expression peaks in different brain regions including neocortex. We further investigated OTR expression patterns in OTR heterozygote reporter mice (OTR-Venus) and found an overall delayed expression pattern, which may be linked to impaired social behavior in the OTR-Venus mice. We envision that our highly detailed OTR expression map will guide future circuit-based investigations to understand the mechanisms of oxytocin signaling in various behavioral assays.

S3.4. AN OPEN ACCESS TOOL FOR ANALYSIS OF CONNECTIONS IN THE PRIMATE CORTEX

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In primates, visual function is dominated by the pathway that transmits visual information from the retina, via the lateral geniculate nucleus (LGN), to the primary visual cortex (V1). Although lesions of V1 lead to blindness, it is well documented that residual visual function can be retained within scotomas caused by V1 lesions, including (largely subconscious) abilities to locate some types of stimuli, and even to coarsely evaluate their characteristics ("blindsight"). These observations indicate that other thalamic projections can convey retinal inputs directly to the extrastriate cortex, bypassing V1. The exact characteristics of blindsight depend markedly on the age at which the lesion occurs. Patients and monkeys who sustained lesions early in life often show a greater range of abilities than those who had lesions in adulthood, including, in many cases, conscious perception. My laboratory has been investigating the types of physiological changes in subcortical and cortical areas which mediate such outcomes. For this purpose, we have developed a V1 lesion model based on the marmoset monkey, a small new world primate in which the anatomy and physiology of the visual pathways has been well characterised, and which has accelerated development in comparison with macaque monkeys. In this talk I will briefly review the characteristics of the marmoset as an advantageous animal model for studies of primate vision, including plasticity, describe

recent findings on the physiological consequences of V1 lesions at different ages, and briefly report on current lines of work aimed at understanding the full circuitry of the marmoset visual cortex using a neuroinformatics approach.

S4.1. STUDY OF NEUROPEPTIDE FUNCTIONS USING ZEBRAFISH AS A MODEL ORGANISM

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Neuropeptides constitute a large class of signaling molecules in the nervous system of many groups of animals. These peptides are extracellular chemical messengers that act as circulating neurohormones, local co-transmitters, or neuromodulators. Neuropeptides have been well conserved during the course of evolution, indicating their major role as regulators of physiological processes. In our lab, we study neuropeptide functioning using zebrafish as a model organism. One of the more interesting neuropeptides we study is galanin. Galanin is a 29-30 amino acid neuropeptide widely expressed in the central and peripheral nervous systems in vertebrates. It co-exists within neurons with several small-molecule classical neurotransmitters and exerts strong inhibitory actions on synaptic transmission by reducing the release of neurotransmitters. Galanin has been implicated in several higher order physiological functions such as nociception, cognition, feeding, mood, and neuroendocrine regulation, which may be relevant to disease states and clinical therapy. Galanin is a highly inducible neuropeptide, showing distinct up-regulation after pathological disturbance within the nervous system. A significant increase in galanin expression is observed after peripheral nerve injury, inflammation, in the basal forebrain in Alzheimer's disease, multiple sclerosis, and during neuronal development. These early studies suggested that increased galanin concentration might have a trophic influence on nerve repair. Galanin has also been observed in a variety of tumors, where it may affect growth and apoptosis. The above information indicates that galanin is a multifaceted peptide. Although it has been 35 years since its discovery, many of its functions are not fully elucidated. We believe that so far applied research models have some limitations which prevent full understanding of the function of galanin. Therefore, we decided to study the function of galanin using another, not used so far, model – zebrafish. Our previous studies revealed that the zebrafish galanin gene is very similar to that of other studied species. The structure of the zebrafish galanin gene is identical to the gene present in mammals. This strong evolutionary conservation may suggest important and similar roles of galanin in all vertebrates. We already studied the expression of galanin during development and in adult zebrafish. In addition, using antisense oligonucleotides (morpholino), we examined the role of galanin in the development of the nervous system. We also confirmed that galanin regulates blood glucose levels. Our studies in transgenic line with inducible overexpression of galanin revealed that galanin reduced the incidence of seizure-like behavior episodes and their intensity. Recently we generated a CRISPR-cas9 zebrafish mutant that is demonstrated to lack the expression of galanin. We use it, among others, to study the role of galanin in the neuroendocrine system, innate immunity, bacterial infection, and regeneration of the nervous system.

S4.2. IDENTIFICATION OF A NOVEL MECHANISM CONTROLLING NEURAL STEM CELL PLASTICITY IN ALZHEIMER'S DISEASE MODEL OF ADULT ZEBRAFISH USING SINGLE CELL TRANSCRIPTOMICS

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Neural stem cells (NSCs) – the reservoir for new neurons – can be harnessed for stem cell-based regenerative therapies in the human brain. In Alzheimer's disease (AD), production of new neurons is suppressed due to hampered proliferative and neurogenic ability of NSCs. Therefore, understanding how the plasticity of NSCs could be induced would be important for designing stem cell-based therapies for AD. Zebrafish have a remarkable ability to regenerate the brain as they can induce NSC plasticity. However, it is still unknown whether the NSC population in the zebrafish brain is heterogeneous and different subtypes could respond differently to disease pathology. We recently identified that NSCs enhance their proliferation and neurogenic outcomes in an Amyloid-beta42-based (Aβ42) experimental AD model in the zebrafish brain and Interleukin-4 (IL4) is a critical molecule for inducing NSC proliferation in AD conditions and this regulation is also observed in human NSCs. However, the mechanisms by which Aβ42 and IL4 affect NSCs remains unknown. Using single cell transcriptomics, we determined distinct subtypes of NSCs and neurons in adult zebrafish telencephalon and identified a novel and IL4-dependent crosstalk mechanism that controls NSC plasticity in AD conditions in adult fish brain and human 3D cultures. Our results constitute an extensive set of resource in the AD model of adult zebrafish brain and provide unique insights into how Aβ42 and IL4 affect NSC plasticity and neurogenesis.

S4.3. IMPAIRED COMMISSURAL TRACT FASCICULATION IS RELATED TO INCREASED EPILEPTOGENESIS AND ANXIETY IN THE ZEBRAFISH MODEL OF TUBEROUS SCLEROSIS COMPLEX

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Tuberous sclerosis complex (TSC) is an autosomal dominant disease caused by mutations in genes encoding for TSC1 or TSC2. These proteins form a complex that inhibits mTORC1 signaling, which activates multiple molecular pathways leading to growth and differentiation in neurons. Lack of TSC1-TSC2 functional complex due to mutations results in mTORC1 overactivation and neurodevelopmental syndromes such as epilepsy, intellectual disability, or autism spectrum disorder. To investigate the mechanisms underlying TSC disease, we use zebrafish mutant TSC2vu242. We examined TSCvu242 fish using various behavioral tests and by live brain imaging with light-sheet microscopy. We confirmed that homozygotic TSC2vu242 mutants were lethal at the early larval stage, underscoring the importance of maintaining proper mTORC1 signaling. Also, TSC2vu242 brains showed increased activation of the mTORC1 pathway and white matter thinning. We discovered improper axon development and axonal tract fasciculation, together with changes in the expression of genes involved in axon guidance. Live imaging showed neuronal hyperexcitability in the brain and epileptogenesis in the early development of the Tsc2-deficient fish. Together with decreased locomotion of TSC2vu242 mutants, these results suggest non-motor seizures. We also examined fish by multiple anxiety-testing behavioral tests and found an increase in anxiety in mutant fish. Moreover, we could rescue anxiety-related behavior and white matter thinning in the TSC2vu242 mutants using the same drug. These results suggest that white matter disruption contributes to neurodevelopmental syndromes such as anxiety and epilepsy in the fish model of TSC disease.

S4.4. HOW ZEBRAFISH RESEARCH IS RESHAPING TODAY'S NEUROSCIENCE AND BIOLOGICAL PSYCHIATRY

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The zebrafish (*Danio rerio*) has become a new powerful model organism in biomedical research. Zebrafish possess all major neurotransmitter receptors, transporters, and enzymes, as well as, express a rich behavioral repertoire, thereby offering a wide spectrum of CNS disease models. However, our understanding of the role of zebrafish as a new emerging mainstream model in neuroscience research is still limited, resulting in incomplete utilization of its several major advantages: 1) phenotypic robustness, 2) ease of experimental manipulations, and 3) high-throughput potential. Today, zebrafish complement other model systems in studying aggression, social interaction, and affective states. Here, I will summarize recent developments made using zebrafish models for the robust elucidation of the genes, molecules, and neurocircuits involved in behavior. Zebrafish possess substantial genetic homology to humans, and an easily manipulated genome to probe genetically-encoded behavior during development and in adulthood. Zebrafish models have contributed to our understanding of the effects of psychoactive drugs and produced innovative brain imaging tools and genetic models that are unique to zebrafish. Collectively, the utility of genetic, pharmacological, and behavioral manipulations in the zebrafish model allows for modeling of crucial brain disorders and diseases. I will highlight the progress made using zebrafish models and the promise they hold for future research to the wider neuroscience field at large. I will argue that zebrafish models occupy a vital niche within the neuroscience field, and when combined with more established rodent and primate models, zebrafish facilitate a more detailed understanding of the biological underpinnings of behavior. I will also provide a framework for the expansion of zebrafish research within translational neuroscience, with the goal of providing novel treatments for brain diseases and an increased understanding of the nervous system.

S5.1. GLOBAL PROTEOMIC ANALYSIS OF BRAIN CHANGES INDUCED BY 'LIFESTYLE' MODIFICATIONS

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In the pathogenesis of central nervous system (CNS) disorders, an increasingly important role is attributed recently to unhealthy lifestyle, which consists primarily of a high caloric diet (i.e., western), chronic exposure to stress, and lack of physical activity. However, the mechanisms responsible for energy metabolism impairment induced by unhealthy lifestyles compromising CNS functions are poorly understood. Research on the effects of physical activity on the CNS is especially important, because it may result in the development of new methods of therapy inspired by natural protective mechanisms. In our study we employed a new and rarely used approach –

a forced running wheel. The lack of electrical stimulus in the aforementioned system successfully makes a breakthrough in the study of animal physical activity. Physiological and behavioral responses of the organism to stress are closely related to sex. Epidemiological studies indicate that women are more vulnerable to the adverse effects of stress and despite that, most of the experimental studies are conveyed on male animals. The investigations were carried on female rats. The main goal of our study was to verify the hypothesis that regular exercise may reduce the disturbances induced by lifestyle modifications, like western diet and/or stress exposure. Adult female rats were fed with the prepared chow reproducing the human western diet and/or subjected to a stress induced by social instability. This stress protocol is characterized by a low degree of invasiveness. To evaluate if regular physical activity may reduce the adverse effects caused by diet and stress, female rats were additionally subjected to the procedure of forced physical activity. A proteomic analysis was conducted on samples obtained from the frontal cortex – a region that plays an important role in cognitive processes as well as is involved in the mechanisms engaged in the response to stress.

S5.2. WHY AND WHAT DO WE EAT OR BRAIN-BODY METABOLIC GAMES

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Eating behavior of animals is controlled by neuronal circuits in the brain, mainly located within the hypothalamus. Hunger is induced by physiological signals, e.g., leptin, informing the brain about energy storages/deficits in adipose tissue. Additionally, other non-physiological factors may influence when and what we eat. Those factors include sensory cues of especially palatable food or are entrained by circadian rhythm. Regulation of activity of neurons involved in the control of feeding and metabolism is achieved on many levels of gene expression. We are especially interested in post-transcriptional level of protein translation regulated by microRNAs. These short RNAs serve as a guides for the translation-inhibiting complex RISC. We have generated transgenic mice with a mutation of the Dicer gene restricted to forebrain neurons of adult mice. The Dicer nuclease is an essential enzyme in the biogenesis of microRNAs. Mice lacking the Dicer gene in the arcuate nucleus of the hypothalamus developed an obesity phenotype due to increased feeding of regular chow diet. We have also examined how different diets – including the standard, high fat diet, Western diet and the ketogenic diet – influence microRNA levels in the blood and preference of mice towards consumed diet. Moreover, we tested cognitive performance of mice fed different diets.

S5.3. NOVEL INSIGHTS OF BRAIN INSULIN ACTION ON METABOLISM AND BEHAVIOR

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The consumption of a high fat diet, which contains mainly saturated long chain fatty acids, induces obesity and insulin resistance in the brain. Insulin resistance in the hypothalamus alters mitochondrial stress responses, specifically the integrated stress response and mitochondrial unfolded protein response (mtUPR) and causes mitochondrial dysfunction and excessive autophagy. Interestingly, reduced expression of genes of the mtUPR is sufficient to induce insulin resistance, highlighting the close interplay of insulin signaling and mitochondrial function in the hypothalamus. Metabolic consequences of brain insulin resistance are hyperphagia and obesity, establishing a vicious cycle for weight gain. Insulin action in the brain does not only impact metabolism but also alters cognition and behavior. Mice with a neuronal insulin receptor knockout (NIRKO) exhibit pronounced anxiety and depressive-like behavior while aging, emotional behaviors which also exist in type 2 diabetic patients. The depressive-like behavior is due to impaired dopaminergic signaling, as insulin receptor deficiency causes elevated monoamine oxidase expression in the mesolimbic system and reduces dopamine half-life. This behavioral phenotype is also true for mice with a specific knockout of insulin receptor in glia cells, highlighting the importance of proper brain insulin action for mood and emotionality. Overall these data demonstrate the crucial role of brain insulin signaling for mitochondrial function, metabolism, and behavior.

S5.4. DIET AS A PREVENTION STRATEGY FOR NEURODEGENERATION DURING AGEING

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Dementia is a global public health concern due to increasing prevalence, high morbidity, and rising socioeconomic burden. Modifying dietary behaviour could be a promising way to enhance cognition and delay or prevent dementia in later life. Several dietary factors influence dementia risk in humans, for example, vitamin E, B vitamins, omega-3 fatty acids and healthy dietary patterns, particularly the Mediterranean Diet, have been shown to be neuroprotective, while high intake of saturated fat accelerates cognitive decline. It

is not entirely clear how diet offers neuroprotection, but several putative mechanisms include beneficial effects on neuronal cell signalling, vascular, anti-oxidant and anti-inflammatory biological pathways. Given that the pathophysiological changes of dementia accumulate years before cognitive impairment becomes evident, understanding the influence of diet on brain health across the life-course is important to inform prevention strategies. Further research is needed to investigate diet-associated neurological change from the earliest through to latest stages of cognitive decline. Furthermore, intervention strategies require insight into mechanisms involved in diet-induced cognitive change and an understanding of how to support dietary behaviour change, particularly in high risk populations.

S6.1. NETWORK ACTIVITIES AT THE START OF A FOCAL SEIZURE

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Abnormally enhanced excitation is commonly believed to mark the onset of a focal seizure. This notion, however, is not supported by any firm evidence and it will be challenged here. We know in fact that a marked reduction of unit firing occurs at the onset of seizures recorded during presurgical intracranial monitoring in patients with focal, drug-resistant epilepsies. Moreover, seizures in animal models of focal epilepsy start with an increased activity of inhibitory interneurons that silences principal neurons. This key, yet paradoxical, role of inhibition in seizure onset has been confirmed in *in vitro* studies where the synchronous activation of $GABA_A$ receptors at seizure onset causes sizeable elevations in extracellular potassium via post-synaptic potassium-chloride co-transport, thus facilitating neuronal recruitment and seizure progression. The proposed role played by inhibition in focal seizure onset should profoundly transform our interpretation of focal ictogenesis thus leading to new antiepileptic strategies.

S6.2. THE TRANSITION TO SEIZURE IS CHARACTERIZED BY THE PROGRESSIVE LOSS OF THE BRAIN'S RESILIENCE

Premysl Jiruska[1,2], Jan Kudlacek[1,2], Jan Chvojka[2,3], Mark J. Cook[4], Dean R. Freestone[4], Philippa J. Karoly[4], Matias I. Maturana[4], John G.R. Jefferys[5], Jakub Otahal[2], Wei-Chih Chang[6]

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To understand seizures and the enduring predisposition of the brain to generate seizures, it is crucial to elucidate the dynamical pathways through which the brain reaches the seizure state. Results from computational modelling studies have predicted the existence of specific pathways to seizure. In our study, we have explored whether the transition to seizure follows principles of a universal dynamical process in nature – critical slowing.

Seizures were induced in rat hippocampal brain slices by perfusion of the slices with high potassium artificial cerebrospinal fluid. Chronic epilepsy in rats was induced by the injection of a minute dose of tetanus toxin to the right dorsal hippocampus. In all preparations, we recorded spontaneous or electrically induced brain activity. The local field potentials were analyzed and temporal profiles of early warning signals of critical slowing determined. Long-term human intracranial recordings were obtained from patients with an implanted seizure monitoring device. Combining the experimental approaches in *in vitro* and *in vivo* models of seizures with the analysis of long-term recordings in patients, we were able to demonstrate that the transition to seizure is associated with changes in neuronal and network activity which displays features of critical slowing – a dynamical phenomenon which reflects progressive loss of brain stability. With approaching seizure, the brain became more sensitive to internal and external perturbations, and it was also characterized by delayed recovery from the perturbations. Our results suggest that transition to seizure may be a slowly changing process characterized by discrete changes in brain dynamics. Also, the loss of resilience has the potential to be better controlled than the random occurrence of stochastic perturbations initiating a seizure.

S6.3. PATHOLOGICAL SYNCHRONIZATION: BIOMARKERS AND NETWORKS IN HUMAN EPILEPSY

Urszula Malinowska

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Surgical resection of the epileptogenic zone (EZ) is the only effective treatment for many drug-resistant epilepsy patients, however the pre-surgical identification of the EZ is very challenging. Unfortunately, it is not possible to precisely define the EZ in practice because the EZ does not have a clear biomarker. For a long time in a history of electroencephalography, sharp, rapid spikes of high amplitude during the ictal period (also occurring interictally) were considered as an epileptiform activity, which indicates the seizure onset zone and was recommended for resection. Recently, high-frequency oscillations (HFO) have been

considered as pathological phenomena correlated with seizure onset zone with higher reliability than interictal spikes and MRI structural lesions. However, oscillations in the frequency range of HFO can also be recorded from normal, non-pathological tissue. To separate the seizure onset zone from the non-pathological area, we proposed a measure of the strength of coupling of high-frequency oscillations with low-frequency activity during seizure onset. All these biomarkers will be discussed and compared with respect to the surgical benefit of patients' outcome.

S6.4. NEURONAL AND IONIC MECHANISMS OF FOCAL SEIZURES – INSIGHT FROM IN SILICO STUDY

Piotr Suffczyński

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It is generally considered that epilepsy and seizures are related to alteration in neuronal excitation/inhibition balance. On the other hand, using an *in vitro* isolated guinea pig brain model of focal seizures, it has been shown that seizures start with strong firing of inhibitory interneurons, silence of principal cells, and a massive increase of extracellular potassium concentration. In order to investigate the link between ionic dynamics and experimentally observed seizure pattern, we developed a computational model of hippocampal network embedded in the extracellular space with realistic Na^+, K^+, Cl^-, and Ca^{2+} dynamics, glial cells, and a diffusion mechanism. The model exhibits seizure-like activity that is qualitatively similar to experimentally observed seizures in the isolated guinea pig brain. We show that, in the model, strong discharge of inhibitory interneurons leads to long lasting accumulation of extracellular potassium, which triggers and sustains abnormal discharges of the neuronal network, including ictal bursting. Using computational modeling, we also suggest novel antiepileptic therapies targeting potassium regulation systems.

S7.1. ELAV PROTEINS AND NEURODEGENERATION: WHICH ROLE IN ALZHEIMER'S DISEASE?

Marialaura Amadio

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Increasing evidence highlights loss of RNA homeostasis as a central feature of many pathological states, including neurodegenerative diseases. The post-transcriptional control of gene expression and its role in neurodegenerative pathologies are gaining increasing attention. Among RNA-binding factors, ELAV proteins are master regulators of many cellular functions by influencing the RNA metabolism (from splicing to translation) of genes with crucial roles in various physio-pathological contexts. Several ELAV-target transcripts are linked to synaptic plasticity, stress response, survival, and proliferation. Evidence from our and other laboratories showed that neuronal ELAV (nELAV) members control gene expression in memory formation, and their alteration may contribute to cognitive impairment associated with Alzheimer's disease (AD). We found that the content of nELAV proteins is significantly decreased along with clinical dementia progression in the hippocampi of AD brains, where it inversely correlates with the amount of amyloid-β (Aβ). In turn, ELAV can affect Aβ precursor protein (APP) processing. The contribution of ELAV members to the neuronal homeostasis and their alteration in neurodegeneration (such as in AD) will be discussed in light of a wider, more complex proscenium where the post-transcriptional (f)actors (e.g. different RBPs, coding and non-coding RNAs) play their role determining the fate of a given transcript.

S7.2. ELAV PROTEINS IN THE PATHOGENESIS OF MOTONEURON DISEASES

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Awaiting for submission.

S7.3. INCREASED INTRAOCULAR PRESSURE ALTERS THE CELLULAR DISTRIBUTION OF HUR PROTEIN IN RETINAL GANGLION CELLS

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Glaucoma is a group of progressive optic neuropathies that lead to irreversible loss of retinal ganglion cells (RGC); the disease can be characterized by several symptoms with a common feature of the visual field loss. Until now, various risk factors of glaucoma development have been identified, but the detailed biological basis of this disease has remained unclear. It has been postulated that the efficiency of cellular endogenous neuroprotective systems can be one of crucial factors affecting the RGC's apoptotic susceptibility. More recently, *in vivo* evidences revealed that changes in HuR subcellular localization within RGCs occurred at early times after IOP induction in an animal model of glaucoma; these effects were followed at longer times by a progressive decrease of cytoplasmic HuR levels, in-

cluding the expression of proteins essential for cell homeostasis (p53, Hsp70) and likely contributes to chronic IOP-induced RGC degeneration. Similar alterations in HuR content and subcellular localization were found in human POAG samples, in support of the involvement of HuR in glaucoma. The potential of HuR as a new pharmacological target is shown by an increasing interest in medicinal chemistry by the field. The role of post-transcriptional mechanisms controlling gene expression in glaucoma, needs to be further explored.

S7.4. ORGANOTYPIC RETINAL EXPLANTS MODEL FOR EVALUATION OF NEUROTOXICITY AND NEUROPROTECTION – THE IMPACT OF METALLOTHIONEIN TREATMENT ON HUR PROTEIN CONTENT

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Retina and optic nerve diseases are one of the major causes of irreversible blindness worldwide, with increasing prevalence associated with aging of the population. Since there is no fully reliable and successful method for culturing retinal neurons (i.e., retinal ganglion cells, RGC) and furthermore, *in vivo* studies are relatively costly and time consuming, the *ex vivo* organotypic retinal culture could be a competitive and highly efficient method for initial drug toxicity screening. Neuroprotection and neuroregeneration are topics of a growing number of studies regarding eye diseases. Ocular pathologies result in neurodegeneration primary or secondary involving RGCs. Metallothioneins (MTs) are low molecular weight cysteine rich proteins. It is suggested that MTs, especially the isoform 2, are important neuroprotective substances for cerebral ischemia and retinal diseases. MT2 as a secondary antioxidant cooperates with reduced glutathione in the cellular protective system against oxidative stress. Additionally, it has been demonstrated that MT treatment can alleviate neurodegeneration of RGC in retinal explants exposed to toxic concentration of gentamycin. This protective effect, linked most probably to antioxidant activity, is associated with delayed increase of HuR protein content in retinal explants, which could be considered as a marker of delayed cells stress response.

S8.1. IMPAIRMENT OF SYNAPTIC PLASTICITY AT IMMATURE GABAERGIC MOSSY FIBER-CA3 SYNAPSES IN ANIMAL MODELS OF AUTISM

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Autism Spectrum Disorders (ASDs) comprise a heterogeneous group of neuro-developmental abnormalities with a strong genetic component, characterized by deficits in verbal and non-verbal communication, impaired social interactions, and stereotyped behaviors. In a small percentage of cases, ASDs are associated with alterations of genes involved in synaptic function. Although rare, these point to synapses as possible sites of ASDs origin. One class of non-syndromic forms of ASDs has been found to be associated with mutations/deletions of genes encoding for neuroligins (NLGs). These are postsynaptic adhesion molecules that, interacting with their presynaptic partners neurexins, ensure the cross-talk between pre- and post-synaptic specializations and synaptic stabilization, necessary for maintaining a proper excitatory/inhibitory balance within local neuronal circuits. Here, transgenic mice carrying the R451C mutation of NLG3 ($NLG3^{R451C}$ KI) or lacking NLG3 (NLG3 KO mice), found in some families with autistic children, were used to study GABAergic signaling in the hippocampus at early stages of postnatal development. We hypothesized that activity-dependent alterations in synaptic plasticity processes represent a convergent mechanism underlying neuro-developmental disorders including ASDs. Unlike littermate controls, $NL3^{R451C}$ KI and NLG3 KO pups failed to exhibit LTP following spike time dependent plasticity, a particular Hebbian type of learning, at immature hippocampal mossy fiber-CA3 synapses, known to express, at early developmental stages, a GABAergic phenotype. These results were associated with a dysfunction of BDNF/TrkB signaling and could be rescued by exogenous application of BDNF. These data clearly show that an early dysfunction GABAergic signaling leads to alterations in the functional refinement of developing circuits and synaptic plasticity processes possibly underlying cognitive deficits in autistic children.

S8.2. SPATIAL REGULATION OF COORDINATED EXCITATORY AND INHIBITORY SYNAPTIC PLASTICITY AT DENDRITIC SYNAPSES

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Traditionally, plasticity was considered to belong mostly to excitatory synapses while inhibitory transmission was assumed to be relatively invariant. However, recent evidences demonstrate several types of inhibitory synaptic plasticity, raising the important question of how GABAergic and glutamatergic synaptic plasticity are coordinated during neuronal ac-

tivity. Here, we found that non-Hebbian postsynaptic depolarizations of principal cells induced inhibitory postsynaptic long-term potentiation (iLTP) in hippocampal cultures. Interestingly, the same protocols induced depression at glutamatergic synapses (LTD), thus indicating an anti-homeostatic relation between inhibitory and excitatory synaptic plasticity. Photolysis of caged glutamate or caged GABA revealed that the aforementioned glutamatergic LTD and GABAergic iLTP are expressed postsynaptically. Subsequently, we investigated how synaptic plasticity induced at individual glutamatergic spines affects the strength of neighboring GABAergic synapses. To this end we induced "single spine LTP" by pairing the postsynaptic depolarizations with repetitive glutamate uncaging at individual spines while simultaneously measuring the strength of adjacent dendritic GABAergic synapses by GABA uncaging. Interestingly, we found that, following the delivery of this hebbian-like protocol, GABAergic synapses located within 3 micrometers from a stimulated spine showed depression (iLTD), while further synapses still showed iLTP. This "spread" of heterosynaptic plasticity from spines was dependent on the protease activity of calpain induced by calcium influx through L-type voltage gated calcium channels. Our findings suggest that both glutamatergic and GABAergic synaptic plasticity are finely coordinated at dendritic level suggesting that the dendritic E/I ratio can be selectively tuned in spatially restricted dendritic sub-regions.

S8.3. LEARNING-EVOKED MODULATION OF GABAERGIC INTERNEURONS INTRINSIC EXCITABILITY IN THE NEOCORTEX

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Somatostatin-expressing interneurons (SST INT) are one of the types of GABAergic neurons in the brain. Inhibition through SST INT is a powerful potential mechanism for gain control in cortical networks, and it has been extensively investigated in studies on learning and memory mechanisms. Learning-related intrinsic excitability changes of SST interneurons have been recognized in the hippocampus. The aim of the study was to analyze how associative learning influences on SST INT-mediated inhibition in the somatosensory cortex of mice. Using a transgenic mouse line with channelrhodopsin expressed in SST cells, we studied SST INT-mediated inhibition onto excitatory neurons, whereas using a transgenic line with fluorescently labeled SST INT, we analyzed intrinsic excitability of SST neurons. The associative learning protocol consisted of whisker stimulation paired with a tail shock (classical conditioning). As control groups, we used naïve mice and mice subjected to stimulation of vibrissae and a tail shock given at random relative to whisker stroking (pseudoconditioning). After learning protocols, we prepared acute brain slices and performed whole-cell patch-clamp recordings in excitatory neurons or SST INT of layer IV in the cortical representation of the whiskers stimulated during learning. Our experiments show that the charge transfer of inhibitory postsynaptic currents evoked in excitatory cells in response to the optical stimulation of SST INT is larger in the conditioned group of mice in comparison to controls. Also, intrinsic excitability of layer IV SST interneurons increases after the conditioning paradigm. Presented data indicate that associative learning increases SST INT-mediated inhibition of excitatory neurons in the somatosensory cortex. The enhancement of this inhibition might rely on the increment of intrinsic excitability of SST cells.

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S8.4. GABAERGIC HIPPOCAMPAL PLASTICITY CRITICALLY DEPENDS ON MATRIX METALLOPROTEINASE 3

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Matrix metalloproteinases (MMPs) are known to play a crucial role in neuronal plasticity. In particular, MMP-3 has been reported to be involved in glutamatergic plasticity and related cognitive processes. Recently, a growing body of evidence indicates that GABAergic synapses are also plastic, but the underlying mechanisms remain elusive. Herein we addressed the question if the activity of MMP-3 is involved in GABAergic synaptic plasticity in mice acute hippocampal slices or in neuronal cultures. The presentation at symposium aims to offer an overview of experimental evidence obtained by our research group while more details will be presented at the posters. We performed whole-cell patch-clamp recordings of miniature inhibitory postsynaptic currents (mIPSCs) from hippocampal CA1 pyramidal neurons. To induce inhibitory LTP (iLTP), we applied NMDA in bath solution (3 min, 20μM) in the presence of 20 μM DNQX and 1μM TTX to slices or neuronal cultures from wild-type (WT) animals and mice lacking *mmp-3 gene* (MMP-3 KO). To block the activity of MMP-3 we used inhibitor UK 356618 (2 μM). Besides functional manifestations, iLTP induction was associated with a significant increase in

synaptic gephyrin cluster area. We found that, in both slices and neuronal cultures, iLTP evoked in MMP-3 KO mice was completely abolished in contrast to WT. An analogous effect was observed when using UK356618. Interestingly, administration of active MMP-3 to neuronal cultures resulted in iLTP and an increase in average size of synaptic gephyrin cluster. In addition, analysis of membrane mobility of synaptic GABAARs showed a decrease in their diffusion coefficient after MMP-3 treatment indicating a strengthening of inhibitory synapses through receptor trapping. We show that the activity of MMP-3 plays a crucial role in iLTP in the hippocampal CA1 region.

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S9.1. MOLECULAR BIOMARKERS OF EPILEPTOGENESIS IN A GENETIC MODEL OF EPILEPSY – TUBERUS SCLEROSIS COMPLEX

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The aim of EPISTOP was to better understand the pathophysiology of epilepsy and its consequences, to develop a preventative strategy for epilepsy, to identify new biomarkers of epilepsy, and to develop new therapeutic targets to block or otherwise modify epileptogenesis in humans. This aim was achieved by a multidisciplinary, systematic approach. First, a prospective study of epilepsy development was conducted in infants with tuberus sclerosis complex (TSC), using a wide range of clinical, neuroimaging, and genetic analyses, including a diverse set of cutting edge analyses of blood samples, at the onset of epileptiform discharges on EEG, at seizure onset and at the age of 24 month. Second, we performed an analysis of biomarkers of epileptogenesis and drug-resistant epilepsy in epileptogenic brain specimens obtained from patients with TSC who underwent epilepsy surgery and TSC autopsy cases collected in the past.

S9.2. MICRORNAS IN EXPERIMENTAL AND HUMAN TEMPORAL LOBE EPILEPSY: BIOMARKER AND THERAPEUTIC POTENTIAL

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MircoRNAs (miRNAs) are small non-coding RNAs that are crucially involved in the post-transcriptional control of gene expression. A number of studies have demonstrated differential expression of miRNAs in the epileptogenic human brain and in blood. Furthermore, changes in miRNA expression are also evident during the development of epilepsy in various animal models for temporal lobe epilepsy. During this presentation, I will highlight specific miRNAs and focus on their biomarker and therapeutic potential.

S9.3. MICRORNAS IN EXPERIMENTAL POST-TRAUMATIC EPILEPSY: BIOMARKER AND THERAPEUTIC POTENTIAL

Nora Puhakka

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Traumatic brain injury (TBI) encompasses primary brain damage inflicted immediately at the time of impact, and secondary damage involving cellular and molecular alterations progressing over a prolonged time post-trauma. Despite a large number of previous successful preclinical proof-of-concept studies, some of which have progressed to failed clinical trials, there are no pharmacotherapies in clinical use that can improve structural and functional recovery from TBI. Early identification of patients at risk of developing the long-term comorbidities is a key aspect for future drug trials testing novel recovery-enhancing and anti-epileptogenic treatments for TBI. We hypothesize that circulating miRNAs can diagnose ongoing epileptogenesis and serve as prognostic biomarkers for post-traumatic epilepsy and behavioral and cognitive co-morbidities in a clinically relevant rat model of TBI. Described research is part of the EPITARGET Consortium.

S9.4. CIRCULATING MICRORNAS AS BIOMARKERS OF EPILEPTOGENESIS/EPILEPSY

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Epilepsy frequently develops as a result of brain insult, e.g., brain injury, stroke, inflammation, or status epilepticus, however currently there are no tools allowing us to predict which patients suffering from trauma will eventually develop epilepsy or how severe it is going to be. In recent years, small non-coding RNAs are proposed as biomarkers for neurological diseases. Particularly microRNAs are interesting candidates, as several of them were described as changing its levels in the brain of epileptic patients and in

epilepsy animal models. There is evidence suggesting that microRNAs levels are also altered in the blood of epileptic subjects, making them attractive candidates for peripheral biomarkers of epilepsy. Levels of miRNA in the blood are altered following epileptogenic stimulus and differentiate between animals with frequent and rare seizures. miRNA may become a useful peripheral biomarker of epileptogenesis/epilepsy as well as severity of the disease. Described research is part of the EPITARGET Consortium.

S10.1. ELECTROPHYSIOLOGICAL PROPERTIES OF FUNCTIONALLY IDENTIFIED ADULT MOUSE MOTONEURONS

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A salient feature in Amyotrophic Lateral Sclerosis (ALS) is that the vulnerability of motoneurons depends on the physiological type of their motor unit. The most vulnerable motoneurons, and the first to degenerate, are the motoneurons of the largest motor units that innervate the fast-contracting and fatigable muscle fibers (FF type), followed by the motoneurons of intermediate motor unit size, which innervate the fast-contracting and fatigue-resistant muscle fibers (FR type). Finally, the motoneurons of the smallest motor units, which mainly innervate the slow contracting and fatigue-resistant fibers (S type) are the most resistant and the least affected at the end-stage of disease. However, not much is known about the physiology of motor units in mice, compared to other species (cats and rats in particular). I have spent the last few years studying the intrinsic properties of adult mouse motoneurons while recording simultaneously the force developed by their motor unit. We've already demonstrated that the unusual electrical properties of mouse motoneurons have a profound impact on the force recruitment of their motor units. In my talk, I will present recent efforts in characterizing the electrical and mechanical properties of type-identified mouse motoneurons and motor units, and their potential relevance for understanding the pathophysiological mechanisms of ALS.

S10.2. ESTIMATING THE TRANSFER FUNCTION OF RAT MUSCLE UNITS

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Awaiting for submission.

S10.3. THE MECHANOMYOGRAM – A VALUABLE TOOL TO ANALYZE MECHANICAL ACTIVITY OF THE MUSCLE

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In the present study, the activity of isolated motor units (MUs) in the rat soleus (SOL) muscle was evoked by stimulation of thin filaments of ventral roots using constant and irregular frequency stimulation patterns. The MUs force, action potentials, and mechanomyograms (MMG) were recorded. MMG profiles were recorded with a laser distance sensor (LDS), categorized and compared with profiles obtained in a similar experiment performed on the medial gastrocnemius (MG) muscle. The profiles and amplitudes of the MMG signal vary greatly depending on the type of stimulated MU, contraction, and LDS localization. Compared to previously obtained results for MG, where three general types of MMG signal were distinguished, in the case of SOL the signal polyphasic signal profile was observed for weak contraction. The steady-state MMG-contraction force relationship could be successfully approximated with a third-order polynomial model. Nevertheless, the model parameters were not constant and changed with stimulation type. The observed phenomena were also analyzed with a 3D model utilizing the Finite Element Method. *In vivo* and simulation results suggested that MMG was an effect of superposition of several movements types evoked by contraction (muscle belly rotation, transverse shearing due to non-axial localization of MUs, local surface deformation). The proposed model set to explain the most likely origins of differences in the MMG profile between MG and SOL muscles. These observations were used to create a novel method of transcutaneous MMG measurement, based on 9-degree of freedom inertial sensors. The technique was applied to the classification of 6 hand gestures based on the MMG signal.

S10.4. SAG IN MOTOR UNIT UNFUSED TETANUS: EXTRA FORCE PRODUCTION AT THE BEGINNING OF ACTIVITY

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Sag is an unexplained property of fast motor units (MUs) and skeletal muscles, and its presence (in fast)

or absence (in slow MUs and muscles) is used in fast/slow twitch recognition. Several series of experiments aimed to identify in the rat muscle factors that contribute to sag, i.e., an extra force production within first 100-300 ms of activity in two types of fast MUs: fast fatigable (FF) and fast resistant to fatigue (FR). First, mathematical decomposition of sagging tetanic contractions of FF and FR MUs into twitch-shape responses to consecutive stimuli was performed. This method identified mechanisms of the sag, including a progressive increase in the amplitude of a few initial responses (a process shorter for FF and longer for FR MUs), followed by a decrease in the amplitude of later responses. In comparison to the first twitch, the relative increase in force amplitudes of the several subsequent decomposed responses was smaller, and their contraction and relaxation times were shorter for FF than for FR units, which corresponded to observed differences in sag profiles between FF and FR MUs. In other series of experiments, effects of occlusion of the blood circulation was studied and these experiments revealed that, under ischemic conditions, the sag disappeared, but it reappeared after restoration of the blood supply. Moreover, we have found a very high sensitivity of the sag amplitude to preceding activation, even for single twitches.

S11.1. HOW CERTAIN ARE CERTAIN "CERTAINTIES" ABOUT DA NEURONS?

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Our knowledge about the dopaminergic system is very wide. It includes both a myriad of minor facts as well as many milestone findings. This knowledge is the basis of theories created and the fuel for further research. However, in view of the increasing complexity of the emerging image of the dopaminergic system, we should verify whether some of the facts that have been taken for granted for decades are not too simplistic. As an example, I will present two observations recently made in our laboratory. First, the bursting pattern of firing of some dopaminergic neurons does not appear to be dependent on NMDA receptors. There is a population of dopamine (DA) neurons that, in response to the stimulation of cholinergic receptors, shift to a bursting mode of firing. The slow dynamics of the observed phenomenon suggest that it may be the basis of dopamine-dependent maintenance of the prolonged states of increased motivation of the animal. The second observation shows that DA neurons can dynamically change both the parameters and the direction of response to the arriving stimuli, depending on the general state of the brain. Thus, the assumption that DA neurons react stereotypically, with inhibition or excitation to a certain type of stimuli (e.g., aversive sensory), may be too simplistic. When we are dealing with a neural system with such a heterogeneous structure, we should seek to clarify some of our observations in this diversity, instead of trying to adapt to existing knowledge, which is often limited to a homogeneous, simplified image of the dopaminergic system.

S11.2. USING COMPUTATIONAL MODELS TO UNDERSTAND ENDOGENOUS AND EXOGENOUS CONTROL OVER DOPAMINE CELL ACTIVITY: WHEN INHIBITION LEADS TO BURSTING

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Dopamine (DA) neurons in the ventral tegmentum and in the substantia nigra are the main source of DA in the basal ganglia and the cortex. These signals are instrumental to motivated behavior, learning, and movement. DA neurons fire in two modes: (1) tonic firing mode at low frequencies that has been argued to control tonic DA levels in the brain and (2) bursting mode, with high frequency spiking packets. The latter is linked with phasic dopamine release signaling reward predictions. While much work has been done on the mechanisms that lead to the two firing modes and how DA neurons go from one to another, these are far from being fully understood. In order to understand these issues, we take a mathematical modelling approach. We developed a circuit model of the ventral tegmental area (VTA) reflecting activity of the DA and GABA neuronal populations, as well as their afferents. We show how the combination of intrinsic cellular excitability together with the structure of the GABAergic synaptic transmission and the glutamatergic inputs allows to control DA cell firing modes. Notably, I will argue that asynchronous inhibition, balanced with asynchronous excitation ensures tonic DA cells firing. Synchronous inhibition leads to burst firing, with intra-burst frequencies far beyond those supported by simple excitation of DA neurons. Time permitting, I will show how drugs of addiction may modify the firing properties of DA neurons.

S11.3. SICKNESS AND MOOD: INFLAMMATORY MODULATION OF DOPAMINE SIGNALING

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Being sick is typically a negative experience. Sickness symptoms like malaise, pain, and nausea have negative emotional components and consequently induce suffering. Whereas the depressed mood and unease induced by sickness might be adaptive under some conditions, they lead to extensive suffering in patients with chronic inflammatory diseases and cancer. We are investigating the routes by which inflammation and other pathological processes control how you feel by regulating dopaminergic signaling. Our studies using cell-type specific genetic modifications and manipulation of neuronal activity show that inflammation-induced aversion is elicited by a long signaling chain involving circulating inflammatory mediators, brain endothelial activation, microglial activation, and prostaglandin mediated modulation of dopaminergic signaling.

S11.4. STRIATAL GABA TRANSPORTER ACTIVITY GOVERNS DOPAMINE TRANSMISSION AND SHOWS MALADAPTIVE DOWNREGULATION IN A MOUSE MODEL OF PARKINSONISM

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Dopamine (DA) release in the striatum is directly gated by mechanisms operating on striatal axons. We recently demonstrated that DA release is under tonic inhibition by a striatal GABA source. Given the paucity of GABAergic axoaxonic synapses on DA axons, this striatal GABA tone presumably arises from ambient GABA. GABA can provide an ambient tone on GABAergic striatal projection neurons at a level limited by striatal plasma membrane GABA transporters (GATs) but whether GATs determine DA output has been unknown.

We reveal that GAT-1 and GAT-3 strongly regulate DA release in mouse striatum by limiting the GABA tone on DA axons in dorsolateral striatum (DLS) but not nucleus accumbens core (NAcC). We find correspondingly greater GAT-1 and GAT-3 levels in DLS than NAcC. Further, we demonstrate that GAT-1 and GAT-3 located at least in part on astrocytes are critical to the level of GABA inhibition of DA release, as astrocyte inactivation prevented the effects of GAT inhibition. Moreover, in a human alpha-synuclein-overexpressing mouse model of parkinsonism, we find that tonic inhibition of DA release by GABA is augmented in DLS but not NAcC as a consequence of decreased GAT levels. Altogether, these data indicate that striatal GATs determine the level of GABA inhibition of DA release in a region-specific manner that supports DA release in DLS, and that GATs are a site of maladaptive plasticity in a model of Parkinson's that limits DA output.

S12.1. INVERSE SYNAPTIC TAGGING OF ARC AND COGNITIVE REFINEMENT PROCESSES

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The neuronal immediate early gene *Arc* plays a critical role in synaptic plasticity and homeostatic scaling by regulating AMPA receptor trafficking. We previously proposed the inverse synaptic tagging mechanism of *Arc* for synapse-specific AMPA receptor regulation. As follow-up studies, we investigated subunit-specific AMPA receptor regulation by *Arc* during structural plasticity in cultured hippocampal neurons. Long-term potentiation (LTP) of surface GluA1/GluA2 levels in spines with volume expansion was undistinguishable between wild-type (WT) and *Arc*-knockout (KO) neurons. However, consistent with the inverse tagging of *Arc* to weak synapses, surface GluA1/GluA2 complex gradually decreased in non-expanding spines of WT neurons during the late phase of structural plasticity, and this effect was abolished in *Arc*-KO neurons. Interestingly, in contrast, LTP of surface GluA2/GluA3 levels showed a significant increase in *Arc*-KO neurons in expanded spines immediately after structural plasticity, while no effect was observed on non-expanded spines. Thus, disruption of *Arc* orthogonally affected distinct of AMPA receptor compositions during early and late phases of LTP in potentiated and non-potentiated spines, without affecting plasticity induction *per se*. These findings strikingly correlated with the normal acquisition yet dysfunctional behavioral refinement in *Arc*-KO mice in two independent target-switching tasks. Network analysis of behavior during the task demonstrated a deficit in target precision in *Arc*-KO mice. Our findings suggest a novel synaptic mechanism by which *Arc* expression regulates cognitive refinement processes such as memory precision and behavioral flexibility.

S12.2. THE ROLE OF ARC/ARG3.1 PROTEIN IN THE REGULATION OF ALCOHOL SEEKING

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Alcohol addiction involves dysregulation of the glutamatergic system. Here, we tested the role of Arc, one of the key regulators of the glutamatergic transmission, in the regulation of alcohol addiction. We observed that Arc KO mice drink as much alcohol as wild-type (WT) animals, but they are more persistent in alcohol seeking during alcohol withdrawal and relapse induced by alcohol-associated cues. Furthermore, we found that Arc protein is upregulated at the synapses of Basolateral (BLA) and Central Amygdala (CeA) (but not DG or CA1 of the hip-

pocampus) in WT mice after withdrawal from long-term alcohol training. To test the function of Arc in the amygdala, we developed and tested gRNAs for Arc knockdown with CrispR/Cas9 system. The most efficient gRNA was introduced on AAV vector together with CrispR/Cas9 into CeA and mice were trained to drink alcohol or sucrose. The mice with local indel mutation of *arc* gene were more persistent in alcohol seeking during cue-induced relapse, had decreased levels of Arc protein in CeA, and increased levels of AMPA receptor subunit GluR2, as compared to the control animals. Local mutation of arc did not affect sucrose seeking and consumption. In conclusion, our data show the novel role of Arc protein in CeA, as a specific regulator of alcohol seeking during relapse induced by alcohol-associated cues.

S12.3. ROLE OF ARC PHOSPHORYLATION IN STRUCTURAL PLASTICITY

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Arc protein was shown to control synaptic AMPA receptor content, dendritic spine maintenance, and structure. Given its importance for neuronal function, Arc protein expression has to be tightly regulated and it occurs via ubiquitination and proteasomal degradation. Glycogen synthase kinases α and β (GSK3α/β) are serine-threonine kinases abundantly expressed in neuronal cells, crucial for neuronal plasticity. GSK3α/β phosphorylate and prime numerous proteins for ubiquitination and degradation, however until now no interaction between Arc and GSK3α/β has been reported. The present study aims to address if and how GSK3α/β affects Arc protein expression, and whether their interaction plays a role in the regulation of dendritic spine morphology.

GSK3-dependent Arc protein degradation and the effects of this process on dendritic spine morphology were studied in cultured embryonic cortical and hippocampal murine neurons upon NMDA receptors stimulation. Arc protein residues modified in GSK3-dependent manner were identified by mass spectrometry. Obtained results were confirmed by *in vitro* kinase assays and the use of anti-phospho-Arc antibodies. We observed higher Arc levels in neurons exposed to NMDA upon GSK3α/β inhibition. *In vitro* kinase assays revealed that Arc is a substrate for GSK3α and β. Further analysis identified four residues phosphorylated by GSK3α/β (S170, T175, T368, T380) and one ubiquitinated in GSK3-dependent manner (K136). Finally, we demonstrated that quadruple phosphodeficient mutant of Arc, as well as ubiquitination-resistant Arc, were more stable in neurons upon NMDAR stimulation, and produced significant thinning of dendritic spine head. Our results identify GSK3α/β-catalyzed Arc phosphorylation and degradation as a novel mechanism for controlling the duration of Arc expression and its effect on dendritic spine structure.

S12.4. THE TEMPORAL DYNAMICS OF ARC EXPRESSION REGULATE COGNITIVE FLEXIBILITY

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Neuronal activity regulates the transcription and translation of the immediate-early gene Arc/Arg3.1, a key mediator of synaptic plasticity. Proteasome-dependent degradation of Arc tightly limits its temporal expression, yet the significance of this regulation remains unknown. We disrupted the temporal control of Arc degradation by creating an Arc knockin mouse (ArcKR) where the predominant Arc ubiquitination sites were mutated. ArcKR mice had intact spatial learning but showed specific deficits in selecting an optimal strategy during reversal learning. This cognitive inflexibility was coupled to changes in Arc mRNA and protein expression resulting in a reduced threshold to induce mGluR-LTD and enhanced mGluR-LTD amplitude. These findings show that the abnormal persistence of Arc protein limits the dynamic range of Arc signaling pathways specifically during reversal learning. Our work illuminates how the precise temporal control of activity-dependent molecules, such as Arc, regulates synaptic plasticity and is crucial for cognition.

POSTER SESSION 1

P1.1. PRENATAL EXPOSURE TO VALPROIC ACID LEADS TO BEHAVIORAL ALTERATIONS AND DEFECTS OF SYNAPTIC PROTEINS IN THE HIPPOCAMPUS OF ADOLESCENT RATS

Agata Adamczyk[1], Magdalena Gąssowska-Dobrowolska[1], Magdalena Cieślik[1], Grzegorz A. Czapski[1], Henryk Jęśko[1], Magdalena Gewartowska[2], Małgorzata Frontczak-Baniewicz[2]

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INTRODUCTION: Autism spectrum disorders (ASDs) are among the most common neurodevelopmental diseas-

es characterized by impairment in communication and social interaction along with stereotyped or repetitive behaviors. Multiple studies have highlighted the involvement of synaptic proteins in the pathogenesis of ASDs.

AIM(S): The aim of this study was to investigate the effect of fetal exposure to valproic acid (VPA) – a rodent model of environmentally triggered autism – on behavioral phenotype as well as gene expression of autism-associated synaptic proteins and synapse morphology in the hippocampus of adolescent rats.

METHOD(S): Pregnant Wistar rats received a single intraperitoneal injection of VPA (450 mg/kg b.w.) on gestational day 12.5. Ultrasonic vocalization was analyzed in all infant rats at postnatal day (PND) 11 and anxiety-related behavior in adolescent male offspring. At PND 52, male offspring were decapitated and the hippocampi were isolated. Transmission electron microscopy (TEM), qPCR, and immunoblotting were used to analyze synaptic structure and protein expression.

RESULTS: VPA administration during pregnancy disturbed communication in neonatal rats and led to anxiety-like and repetitive behavior in adolescent animals. TEM showed synaptic pathology including nerve endings swelling, blurred and thickened synaptic cleft structure, and disruption of synaptic membranes. Ultrastructural changes were accompanied by increased expression of proteins involved in synaptic vesicle recycling and neurotransmitter release (Synaptobrevin, Synaptophysin, Synapsin-1) and reduction in presynaptic membrane protein SNAP25 and the postsynaptic density scaffold PSD95. Changes also occurred in the expression of Shank family proteins and neuroligin 3.

CONCLUSIONS: Deregulated expression of synaptic proteins could be involved in ASDs via alterations of synaptic structure/function, subsequently contributing to behavioral abnormalities.

FINANCIAL SUPPORT: Supported by NSC grant 2017/25/B/NZ4/01969.

P1.2. PRENATAL EXPOSURE TO VALPROIC ACID LEADS TO DEREGULATION OF PURINERGIC SIGNALING IN THE BRAIN OF ADOLESCENT RATS

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INTRODUCTION: Purinergic signaling is involved in many neurodevelopmental alterations that eventually lead to severe disorders, such as autism spectrum disorders (ASD). It was suggested that metabolic pathways that synthesize and catabolize purines are critical regulatory elements in ASD and play a role in promoting behavioral abnormalities. However, the molecular basis of the altered purine metabolism in autism still remains to be elucidated.

AIM(S): Investigation the effect of embryological exposure to valproic acid (VPA) – rodent model of environmentally triggered autism – on extracellular nucleotides level and turnover as well as the expression and activity of selected purinergic receptors in the brain cortex of adolescent rats.

METHOD(S): Pregnant Wistar rats received a single i.p. injection of VPA (450 mg/kg b.w.) on gestational day 12.5. We isolated CSF and brain cortex from the adolescent (52-day-old) male offspring. The experiments were also conducted on primary neuronal cultures isolated from VPA rat pups on the 19th embryonic day.

RESULTS: Prenatal exposure to VPA significantly elevated ATP, ADP, and AMP levels in the CSF of adolescent animals, whereas the level of adenosine was unchanged. Concomitantly, cortical expression of ATP hydrolyzing enzymes was significantly decreased. Prenatal exposure to VPA also generated a rearrangement of selected ionotropic and metabotropic purinergic receptors. While the level of purinergic P2X7 and P2Y1 receptors was decreased, the expression of P2X1 receptor was elevated. Additionally, we observed hyperactivity of purinergic receptors in primary neurons isolated from VPA animals. Stimulation of these cells with 1mM ATP induced a 4-fold increase in intracellular calcium level as compared to control cells.

CONCLUSIONS: Prenatal exposure to VPA induces purinergic signaling deregulation in the brain cortex of adolescent offspring and may be involved in the behavioral deficits in ASD.

FINANCIAL SUPPORT: Supported by NSC grant 2017/25/B/NZ4/01969.

P1.3. EVALUATION OF TRANSGENIC MICE WITH PROGRESSIVE DEGENERATION OF NORADRENERGIC SYSTEM TOWARDS THE STUDY OF PRESYMPTOMATIC PHASE OF PARKINSON'S DISEASE

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INTRODUCTION: Parkinson's disease (PD) is characterized by an inevitable loss of dopaminergic cells.

However, examination of human brain tissues revealed that noradrenergic cell loss in the region of the locus coeruleus (LC) may proceed and may be even greater than dopaminergic degeneration.

AIM(S): The aim of this study was to determine whether genetically evoked, selective loss of LC noradrenergic neurons in a progressive manner may negatively influence the dopaminergic system. Our mice models have progressive degeneration of the noradrenergic system, based on deletion of the gene *Rrn3* encoding transcription factor TIF-IA, which is essential for the regulation of rRNA synthesis.

METHOD(S): First, we applied the conditional inactivation of the *Rrn3* by the Cre-loxP system expressing Cre recombinase under DBH promoter. TIF-IADBHCre mice revealed ptosis, reduced locomotor activity, and a shortened life span associated with enhanced expression of various neurodegenerative markers within the dopaminergic system, including upregulation of micro- and astroglia, pro-inflammatory proteins, and enhanced level of oxidative stress. To limit mutations to the CNS, in a second model a Cre-dependent lentiviral vector carrying the *Rrn3* deletion created by the CRISPR/Cas9 system was directly delivered to LC of DBHCre mice.

RESULTS: Our construct was first successfully tested *in vitro* on primary dopamine neurons followed by *in vivo* stereotactic application. This approach seems to be successful as, in preliminary data, we observed the disintegration of nucleoli in transduced noradrenergic neurons in LC, which is the determinant of the functional impairment of the targeted TIF-IA.

CONCLUSIONS: To-date, there are no experimental studies on possible long-term negative impacts of progressive noradrenergic degeneration on other neurotransmitter systems, despite the clinically observed concomitant loss of SN/VTA and LC neurons in PD. If we provide additional evidence, mice with ongoing neurodegeneration of LC neurons may became a valuable tool for studying the presymptomatic phase of PD.

P1.4. MODIFICATION OF MONOSYNAPTIC IA EPSP BY EXTERNALLY INDUCED ELECTRICAL FIELDS IN SOD1 G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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INTRODUCTION: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a progressive loss of motoneurons with no viable treatment available. A dysregulation of facilitation/inhibition coupling that forces motoneuron hypoexciatability appears to be a key mechanism of the degeneration, and preliminary results suggest that the chemogenetic increase of motoneuron activation ameliorates the disease burden.

AIM(S): Here we propose a novel method of manipulating motoneuron synaptic excitation in the SOD1 mouse model of ALS, using the trans-spinal direct current stimulation (tsDCS) technique, which influences both motoneuron intrinsic excitability and synaptic excitation.

METHOD(S): Experiments were carried out on presymptomatic SOD1-G93A mice. Animals were deeply anesthetized with a mix of fentanyl/medetomidine/midazolam, artificially ventilated, and paralyzed. Intracellular recordings of triceps surae (TS) motoneurons allowed recording of monosynaptic EPSPs from electrically stimulated proprioceptive Ia afferents, which were subsequently conditioned with cathodal tsDCS of 0.1 mA.

RESULTS: Cathodal polarization evoked an acute increase of the Ia EPSP amplitude recorded in TS motoneurons (max 200% of control n=10). These alterations were not matched by changes in the Ia afferent activity or motoneuron passive membrane properties, suggesting that the loci of the effects is restricted to the pre- or postsynaptic elements of the Ia synapse. Interestingly, the effects of polarization outlasted its application by at least 15 min.

CONCLUSIONS: tsDCS is a potent way of manipulating motoneuron synaptic excitation and may play a role as a therapeutic method for managing ALS. However, the influence of this technique on motoneuron intrinsic excitability and disease progression remains to be elucidated.

FINANCIAL SUPPORT: This work was supported by NCN grant no. 2017/26/D/NZ7/00728.

P1.5. MODULATION OF HIPPOCAMPAL THETA ACTIVITY BY TRANSCUTANEOUS STIMULATION |OF TRIGEMINAL NERVE IN ANESTHETIZED RATS

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INTRODUCTION: Neuromodulation is a therapeutic technique that involves modification of neural function via external stimulation, usually in the form of electrical impulses. Prominent examples of neuromodulative therapies include vagus nerve stimulation for epilepsy, Alzheimer's disease, and even deep brain stimulation for Parkinson's disease. Trigeminal nerve stimulation (TNS) has also been applied as a therapeutic tool in a va-

riety of disease processes and has shown efficacy in the treatment of epilepsy and depression.

AIM(S): The aim of the present study was to evaluate if transcutaneous TNS (t-TNS) could affect the hippocampal EEG activity.

METHOD(S): Male Wistar rats were implanted with a tungsten microelectrode for recording hippocampal formation (HPC) field activity. Furthermore, two uninsulated tungsten electrodes were used for t-TNS in eye-ear line in anesthetized rats. The stimulation was conducted at three point: closer to the eye (point 1), in the middle of line eye-ear (point 2), and closer to the ear (point 3). The following t-TNS intensities ware tested: 4, 6, 8, and 10 mA. The remaining parameters of stimulation were constant: pulse duration (1 ms), train duration (10 s), frequency (10 Hz).

RESULTS: Stimulation only in the point closer to the ear and in the eye-ear line was found to be productive in inducing nicely developed and well-synchronized hippocampal theta rhythm. However, t-TNS in the range below 6 mA was found to be subthreshold (theta rhythm was not observed in HPC). Theta activity was observed at point 3 during stimulation only when the intensity of stimulation was equal or higher than 6 mA.

CONCLUSIONS: Only the point located closer to the ear in the eye-ear line was found to be effective in inducing t-TNS theta rhythm. The effectiveness of t-TNS on hippocampal theta rhythm was determined by its intensity and density of trigeminal nerve endings.

FINANCIAL SUPPORT: These studies were supported by The National Centre of Research and Development (grant no. 01.02.00-00-0023/17-001).

P1.6. NEUROPROTECTIVE EFFECTS OF METABOTROPIC GLUTAMATE RECEPTORS GROUP II (MGLUR2/3) AGONISTS IN AN ANIMAL MODEL OF BIRTH ASPHYXIA

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INTRODUCTION: Hypoxic-ischemic encephalopathy (HIE) results in permanent damage of the central nervous system that may result in neonatal death or developmental disorders. 20% – 30% of infants with HIE die in the neonatal period, and 33% – 50% of survivors demonstrate permanent neurodevelopmental abnormalities, such as cerebral palsy and mental retardation. It was shown recently that group II metabotropic glutamate receptor (mGluR2/3) activation before or after ischemic insult results in neuroprotection, but the exact mechanism of this effect is not clear.

AIM(S): The aim of present study was to investigate whether mGluR2/3 activation after hypoxia-ischemia reduces brain damage and if the reduction of the expression of pro-apoptotic factors is one of the mechanisms involved.

METHOD(S): We used an animal model of hypoxia-ischemia (H-I) on 7-day old rat pups. Animals were anesthetized and the left common carotid artery was isolated, double-ligated and then cut between the ligatures. After completion of the surgical procedure, the pups were subjected to hypoxia (7.4% oxygen in nitrogen for 75 min at 35˚C). Control pups were sham-operated (anaesthetized and left c.c.a. dissected, but not ligated). Animals were injected intraperitoneally with specific mGluR2 (LY 379268) and mGluR3 (NAAG) agonists 1 h or 6 h after H-I (5 mg/kg of body weight). The weight deficit of the ischemic brain hemisphere was measured and expression of Bax, Bcl-2, and HTR/OMI was examined. Damage in the hippocampal CA1 region was examined by cresyl violet (CV) staining.

RESULTS: Our results show that application of mGluR2/3 agonists after H-I results in neuroprotection. Both applied agonists decreased brain tissue weight loss in ischemic hemisphere at both times of application (from 40% in H-I to 15% – 20% in treated). Histological examination of the brain tissue showed that both mGluR2/3 agonists applied 1h or 6 h after H-I decreased the damage of neuronal cells and the disorganization of CA1 region of hippocampus. Both agonist mGluR2/3 applied 1h or 6 h after H-I were associated with decreased expression of BAX and HTR/OMI and increased expression of Bcl-2 in the ischemic brain hemisphere as compared to H-I.

CONCLUSIONS: The results show that activation of mGluR2 or mGluR3 in a short time after H-I insult triggered neuroprotective mechanisms and reduced apoptotic processes initiated by H-I in the developing brain.

FINANCIAL SUPPORT: This work was performed under the 2016/23/N/NZ7/01942 project.

P1.7. MATERNAL SEPARATION MODIFIES DENDRITIC SPINE DENSITY IN VENTRAL TEGMENTAL AREA NEURONS IN A SUB-REGION-SPECIFIC MANNER: STUDY IN FEMALE RATS

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INTRODUCTION: Early life stress disrupts development of the human and animal brain and increases the risk of psychophysiological disorders and susceptibility to addiction in adulthood. Maternal separation

(MS), an animal model of early life stress, was shown to raise predisposition to addictive behaviours and change neuronal activity as well as dendritic spine density in a range of brain structures. Mesocorticolimbic dopaminergic pathways originating from the ventral tegmental area (VTA) play a crucial role in the development of addiction, however, the influence of MS on VTA neuronal architecture remains obscure.

AIM(S): The current study aimed to verify the influence of MS on VTA neuronal dendritic spine density, a possible anatomical substrate of functional changes in the ascending dopaminergic pathways.

METHOD(S): Female rat pups were separated from dams for 3 hours daily from PND2 to 14. At PND65, rats were decapitated, Golgi-Cox staining was performed, and the density of spines was calculated manually on I-III- order dendrites. Given the functional and anatomical heterogeneity of the VTA, analyzed neurons were assigned to specified VTA sub-regions.

RESULTS: In rats subjected to MS, significantly lower density of dendritic spines on neurons in ventromedial (II-order branches – 15%), dorsolateral (II- and III-order segments – 24 – 23% respectively), and dorsomedial (I-order branches – 25%) VTA, was observed when compared to control. No significant changes in spine density were observed in ventrolateral VTA (only 6% decrease in spine density).

CONCLUSIONS: The observed decrease in dendritic spine density in VTA neurons can be linked to a reduced number of excitatory synapses that may underlie altered activity of mesocorticolimbic pathways, altered dopamine release, and increased susceptibility to addictions observed after MS and childhood trauma. Sub-region specificity of observed changes points to varied sensitivity of VTA neurons to stress.

FINANCIAL SUPPORT: Funding: NSC-Poland UMO-2016/21/B/NZ4/00204.

P1.8. COMPARISON OF DOPAMINERGIC NEURONS GENERATED FROM IPS CELLS FROM HEALTHY VOLUNTEERS AND PATIENTS WITH THE IDIOPATHIC FORM OF PARKINSON'S DISEASE

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INTRODUCTION: Neurodegenerative disorders are acquired or inherited diseases of the nervous system, in which the main causative mechanism is loss of specific subtypes of neurons. Due to the fact that neurodegeneration processes are poorly understood, present therapies allow only for a delay in the progression or a decrease in symptoms. An interesting approach is modelling of cellular interactions within the human brain using pluripotent stem cells. Induced pluripotent stem (iPS) cells are unique among other cells through their differentiation potential and self-renewal ability, thus they can play a key role in modeling the disease. Recent achievements in differentiation of stem cells enable generation of dopaminergic neurons from human iPS cells.

AIM(S): The aim of this work was to obtain iPS cells from healthy volunteers and Parkinson's disease (PD) patients with the idiopathic form of the disease and to compare dopaminergic neurons derived from iPS cells of both groups.

METHOD(S): To achieve this goal, peripheral blood mononuclear cells were reprogrammed with Sendai virus infection. Generated iPS lines were able to differentiate into the 3 germ layers *in vivo* (teratoma formation assay) and were also positive for alkaline phosphatase. Embryonal markers were evaluated by RT-PCR. The differentiation of human iPS cells into dopaminergic neurons was performed. Expression of neuronal markers on differentiated cells was analyzed by immunostaining and RT-qPCR.

RESULTS: Differences between neurons generated from PD patients with the idiopathic form of the disease and healthy volunteers were observed.

CONCLUSIONS: Moreover, preliminary results show immense potential for further analysis of dopaminergic neurons from patients with idiopathic forms of PD.

FINANCIAL SUPPORT: The project was supported by a grant from the National Science Centre in Poland 2015/17/B/NZ5/00294.

P1.9. DAT1 GENE, ADHD, AND THE DEVELOPMENT OF ATTENTIONAL FUNCTIONS

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INTRODUCTION: Neuropharmacological and human clinical studies have suggested that the dopaminergic system of the brain is substantively involved in normal and pathological phenotypes of attention. Dopamine transporter gene (*DAT1*) was proposed as a candidate gene for Attention-Deficit/Hyperactivity Disorder (ADHD).

AIM(S): To investigate the effect of the *DAT1* gene on performance in the several attentional tasks.

METHOD(S): ADHD and healthy children and teenagers aged 9 – 16 were evaluated using tests and pro-

cedures involving attentional switching, selective and sustained attention (Test of Everyday Attention, TEA-Ch and Sustained Attention to Response Test, SART), and also three attentional networks – alerting, orienting, and executive attention (Attention Network Test, ANT). *DAT1* polymorphism analysis was performed by polymerase chain reaction on saliva samples provided by subjects. ADHD children performed significantly worse in comparison to healthy controls in most of the tasks, demonstrating deficits in various attention processes which were persistent within the examined age range. The results showed an effect of improvement in almost all indices of attentional processes with increasing age in both ADHD and control groups.

RESULTS: The results revealed a significant main effect of *DAT1* genotype for switching, wherein subjects carrying the 9R allele displayed worse performance in comparison to children with 10R/10R and 10R/11R genotypes. A similar effect of genotype was observed for orienting, which was not disturbed in ADHD subjects. No association between ADHD and the *DAT1* polymorphism, and no interaction of *DAT1* genotype and ADHD diagnosis were found.

CONCLUSIONS: *DAT1* is associated with attentional switching and orienting. ADHD is associated with deficits in primary functions that are distinct from those associated with the *DAT1* gene polymorphism.

FINANCIAL SUPPORT: This research was supported by National Science Centre Poland Grants 2011/01/D/NZ4/04958 and 2015/17/N/HS6/03020.

P1.10. CACYBP/SIP AND β-CATENIN HOMEOSTASIS IN THE YAC128 MICE MODEL OF HUNTINGTON'S DISEASE

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INTRODUCTION: We previously detected a 2-fold increase of CacyBP/SIP gene expression encoding calcyclin-binding protein (CacyBP/SIP) and increased CacyBP/SIP dimerization in the striatum of YAC128 mice, a model of Huntington's disease (HD). In these mice, mutated huntingtin is overexpressed in neurons. In agreement with the suggested role of CacyBP/SIP in β-catenin degradation, we observed a decrease in total protein ubiquitination and a higher level of β-catenin in the striatum of HD mice as compared to wild-type animals.

AIM(S): To check if there is a relationship between the level of CacyBP/SIP dimerization in the YAC128 model and β-catenin signaling and neuronal neurodegeneration.

METHOD(S): To impair or increase the ability of CacyBP/SIP to dimerize, mutations in its coiled-coil dimerization domain were computationally designed using the crystal structure as a template and the Rosetta energy function and molecular dynamics methods. Native gel electrophoresis was used to confirm the effects of mutations in dimer stabilization and destabilization. In cultures of medium spiny neurons (MSNs) isolated from the striatum of YAC128 mice, CacyBP/SIP mutants were overexpressed by lentiviruses and used to analyze the level of β-catenin and its ubiquitination by immunoprecipitation and western blotting.

RESULTS: In HD MSNs in which wild-type CacyBP/SIP was overexpressed, the level of β-catenin and its ubiquitination was unchanged relative to the control vector. The effects of mutations leading to increased (K21W and a double mutant T30R, S33E) or decreased ability of CacyBP/SIP to dimerize (D11A, E14A, and L18A) on β-catenin homeostasis are being analyzed.

CONCLUSIONS: CacyBP/SIP protein mutants with different abilities to dimerize allow us to establish the role of dimerization on the β-catenin level, which might be related to HD pathology.

FINANCIAL SUPPORT: Supported by National Science Centre in Poland. Grant no. 2014/15/D/NZ3/05181 to MC.

P1.11. TRAUMATIC BRAIN INJURY-INDUCED CHANGES IN ELECTROPHYSIOLOGICAL PROPERTIES OF GRANULE CELLS AND ADULT HIPPOCAMPAL NEUROGENESIS

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INTRODUCTION: Traumatic brain injury (TBI) affects millions of people, representing a major public health concern. Several important cognitive functions altered by TBI depend on the hippocampus, where new neurons are born throughout life. Functional plasticity of synaptic networks in the hippocampus has been implicated in the development of posttraumatic epilepsy after TBI. The hippocampus dentate gyrus (DG) acts as a "gatekeeper" and "filter" of aberrant or excessive input information. DG function is directly determined by a delicate balance between neuronal excitation and inhibition and TBI can cause changes in this state of equilibrium. It has been postulated that TBI-induced hyperexcitation of preexisting DG granule cells (GCs) can affect adult hippocampal neurogenesis (AHN) and induce

long-term changes in both neural stem cells (NSCs) and newborn neurons, and those alterations can contribute to hippocampal dysfunction.

AIM(S): We aim to understand what particular changes TBI induces at the cellular, molecular, and electrophysiological levels in preexisting GCs, NSCs, and newborn neurons, using a model of controlled cortical impact.

RESULTS: Observed changes in spontaneous excitatory currents (sEPSCs) frequency indicate remodeling of excitatory input, likely expressed as an increase in the number of excitatory synapses. Those changes are accompanied by a decrease in spontaneous inhibitory currents (sIPSCs) frequency, indicating a loss of GABAergic neurons. Moreover, we have observed an increase in neurogenesis up to two months after the injury. These newborn neurons, however, present altered morphology and migration.

CONCLUSIONS: In addition, we have found that NSCs get activated in higher numbers and acquire a reactive-like phenotype that is most likely caused by hyperexcitation.

FINANCIAL SUPPORT: This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska–Curie grant agreement no. 799384.

P1.12. LIPOPOLYSACCHARIDE ACCELERATES NEUROINFLAMMATION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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INTRODUCTION: The amyloid hypothesis postulates that the main cause of Alzheimer's disease (AD) is amyloidogenic cleavage of amyloid precursor protein (APP) and deposition of amyloid-beta. Recently, another hypothesis was formulated that neuroinflammation may precede amyloid generation in AD development. It was also demonstrated that systemic inflammation may impair brain homeostasis and function.

AIM(S): Based on these data we hypothesized that systemic inflammation impairs brain homeostasis and leads to neuroinflammation that later causes AD development.

METHOD(S): To verify this hypothesis, we compared effects of systemic inflammation induced by intraperitoneal injection of lipopolysaccharide (LPS) in transgenic mice expressing human APP with Swedish AD-causing mutation (APP_{swe}) to untreated APP_{swe} mice. To assess AD neuropathological hallmarks, brain tissue from 4, 8, and 12-month old animals were analyzed by immunohistochemical staining and immunoblotting.

RESULTS: We found that LPS shortly after peripheral administration to APP_{swe} mice induced astrogliosis and dysregulation of pro- and anti-inflammatory cytokines in brains already in young 4-month old animals and these effects were also detected in 8-month old mice. In control mice not treated with APP_{swe}, the development of signs of neuroinflammation was slower. We also compared the signs of neuroinflammation in the hippocampus and entorhinal cortex to levels of APP full-length protein and its pathologically truncated CTFs forms.

CONCLUSIONS: Obtained results indicate that systemic inflammation accelerates and intensifies neuroinflammation as reflected by astrogliosis and pro-inflammatory reaction during AD development. It suggests that systemic inflammation can be considered as a common civilization risk factor of AD progression. These data became the reference for the next hypothesis and studies of our group (abstracts by A. Mietelska-Porowska and by A. Więckowska).

FINANCIAL SUPPORT: Financed by National Science Center grants no. 2014/15/D/NZ4/04361, 2018/29/N/NZ7/01724.

P1.13. DIET-DERIVED CHANGES IN INSULIN METABOLISM IN BRAIN ACCELERATE THE DEVELOPMENT OF ALZHEIMER'S DISEASE NEUROPATHOLOGICAL FEATURES

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INTRODUCTION: The western diet (WD), enriched in saturated fatty acids, cholesterol, and simple carbohydrates., is known to cause metabolic syndrome related to insulin metabolism impairment. On the other hand, metabolic syndrome is described as a potential risk factor for Alzheimer's disease (AD). Main early AD features in brain are altered proteolysis of amyloid precursor protein (APP) and hyperphosphorylation of tau protein.

AIM(S): Our aim was to verify our hypothesis that the WD causes insulin metabolism disturbances and may accelerate development of early AD hallmarks.

METHOD(S): To verify this hypothesis, we compared effects of WD feeding (from 3rd month of age) in transgenic mice expressing human APP with Swedish AD-causing mutation (APPswe) compared to APPswe mice in which systemic inflammation was induced by injection of lipopolysaccharide (LPS; the model described in the abstract by J. Dlugosz), and to untreated APPswe mice. To

assess AD neuropathological hallmarks, all groups were analysed at the ages of 4, 8, and 12-months by immunohistochemical and immunoblotting analysis.

RESULTS: Our results demonstrate levels of insulin resistance marker and insulin/Aβ degrading enzyme in relation to characteristic neuropathological AD hallmarks, such as occurrence, intensity of staining, and neuronal compartmentalisation of phosphorylated isoform of Tau protein, and the level of APP full-length protein and its pathologically truncated CTFs forms, in the hippocampus and cortex of mice brains.

CONCLUSIONS: Obtained results indicate that WD is linked to insulin metabolism impairment and leads to accelerated over-phosphorylation of tau protein and proteolysis of APP. This suggests that the WD, via impairment in insulin metabolism, may accelerate the development of AD.

FINANCIAL SUPPORT: Financed by National Science Center grant no. 2014/15/D/NZ4/04361.

P1.14. WESTERN DIET – FROM METABOLIC DISTURBANCES TO ACCELERATION OF GLIA ACTIVATION AND DEVELOPMENT OF ALZHEIMER'S DISEASE

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INTRODUCTION: A diet enriched in fat, cholesterol, and sugar – called the Western diet (WD) – was shown to induce systemic inflammation, obesity, and metabolic syndrome and results directly and indirectly in an impact on brain structure and function. The WD has not been examined yet in the context of Alzheimer's disease (AD), characterised by altered cleavage of amyloid precursor protein (APP) and deposits of toxic amyloid.

AIM(S): We aimed to verify the hypothesis that WD by inducing metabolic syndrome and systemic inflammation may accelerate brain glia activation events and the onset of AD.

METHOD(S): To verify this hypothesis, transgenic mice expressing human APP with Swedish AD-causing mutation (APPswe) were fed with WD from 3rd month of age. These mice were compared to APPswe mice in which systemic inflammation was induced by injection of lipopolysaccharide (LPS) and to untreated APPswe mice. All animal groups were subsequently analysed at the age of 4, 8, and 12-months by immunohistochemical and immunoblotting analysis.

RESULTS: Already one month of WD feeding induces metabolic disturbances including hypercholesterolemia and hyperglycaemia and accelerates the brain pathological events in young APPswe mice. After one month of WD feeding, we observed enhanced astrogliosis, altered profile of microglia activation state, and enhanced cleavage of APP in mice brains. Moreover, we observed obesity, enhanced liver weight, and non-alcoholic fatty liver disease (NAFLD) after 3 months of the WD, which suggest that the WD causes alterations in the brain even earlier than in peripheral organs.

CONCLUSIONS: These results suggest that the WD leads to brain neuroinflammation and accelerates the development of AD. Therefore, the WD can be considered as a newly identified common civilian AD risk factor.

FINANCIAL SUPPORT: Supported by National Science Center grants: 2014/15/D/NZ4/04361, 2018/29/N/NZ7/01724.

P1.15. IDENTIFICATION OF POTENTIAL KEAP1 INHIBITORS THROUGH DATABASE ANALYSIS BASED ON THE DESIGNATED PHARMACOPHORE AND QSAR MODELS

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INTRODUCTION: Oxidative stress is the cause of various pathophysiological conditions, including neurodegenerative diseases. Under physiological conditions, Nrf2 transcription factor is associated with the Keap1 inhibitory protein and anchored in the actin cytoskeleton, and hence its transcriptional activity is limited. The Keap1-Nrf2 regulatory pathway plays an important role in cell protection, therefore the search and design of Nrf2 modulators (inhibitors or activators) becomes a potential therapeutic target. Recent research shows that Keap1 is the key negative Nrf2 regulator, and the development of effective inhibitors that activate the Nrf2 transactivation function by inhibiting the Keap1-Nrf2 interaction is a promising research direction.

AIM(S): Attempt to find Keap1 inhibitors by determining the pharmacophore and analysis of the QSAR models.

METHOD(S): The set of data used in the study was composed of activators collected from the literature. Structures of the data set were drawn using ChemBioDraw ultra 12.0. Crystal structure and retrieved from the Protein Data Bank. The 3D-QSAR pharmacophore model was developed using DS software.

RESULTS: Analysis of Keap1 structural features, including active sites and binding sites, and analysis of the privileged structures of already described inhibi-

tors enabled the generation of a pharmacophore. This allowed us to prepare quantitative structure-activity relationships (QSAR) models. Next, based on the received models, attempts were made to search virtual databases, which resulted in the designation of five potential inhibitors for the Keap1-Nrf2 complex.

CONCLUSIONS: The test effect can be considered satisfactory. Certainly, the analyses carried out were new to the researcher and constitute important grounds for further research. Such analyses provide interesting insights, not only on the problem of Keap1 inhibitors, but also on the use of chemo- and bioinformatic tools in neurobiological sciences.

P1.16. SUBTHALAMIC NUCLEUS DEEP BRAIN STIMULATION INFLUENCE ON ERYTHROCYTE NUMBER AND HEMOGLOBIN LEVELS IN A RAT MODEL OF EARLY PARKINSON'S DISEASE

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INTRODUCTION: Subthalamic nucleus deep brain stimulation (STN-DBS) is most effective treatment for Parkinson's disease (PD) motor symptoms. A number of epidemiological studies have recently highlighted the association between hemoglobin (HGB) levels and PD risk. Interestingly, several lines of evidence confirm that STN-DBS increases regional cerebral blood flow and oxygen concentrations in target brain areas.

AIM(S): Considering the close association between oxygen concentration, red blood number (RBC), and HBG, we hypothesized that enhanced blood flow during STN-DBS may influence peripheral RBC parameters in a rat model of early PD.

METHOD(S): Male Wistar rats were implanted unilaterally for STN-DBS and received intranigral (substantia nigra pars compacta, SNpc) infusion of 6-OHDA. After recovery, rats were subjected to STN-DBS for 7 days (1h daily, n=6) or SHAM stimulation (control, n=6). Immediately after collection, peripheral blood samples were analyzed using automated hematology analyzer (Cell Dyn 3700). The RBC number, hematocrit percentage (HCT), HGB concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured. PD model was verified by the detection of tyrosine hydroxylase positive neurons in SNpc. For a statistical analysis of the results, SPSS 22.0 software was used.

RESULTS: The Student's t-test showed that STN-DBS rats had a significantly higher number of RBC in comparison to the SHAM rats ($t_{(10)}$=-2.912; $p \leq 0.05$). The HCT percentage slightly increased but differences did not reach statistical significance. Mann-Whitney U tests showed that HGB level was higher in STN-DBS rats (Z=-1.290; $p \leq 0.05$).

CONCLUSIONS: The STN-DBS applied in a rat model of early PD has an influence on RBC number and HGB level. The obtained results suggest that there are peripheral compensation mechanisms for the increased oxygen demand during STN-DBS in rats.

FINANCIAL SUPPORT: Department of Animal and Human Physiology fund.

P1.17. SOCIAL PLAY BEHAVIOR OF RATS IN THE MODEL OF AUTISM INDUCED BY PRENATAL EXPOSURE TO POLYINOSINIC: POLYCYTIDYLIC ACID

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INTRODUCTION: Social play behavior is crucial for acquiring social skills and the development of normal socioaffective responses. The repertoire of social play in rats changes throughout the life, peaking during the juvenile period and falling off around adolescence. The most characteristic postures in social plays of rodents are pinning and pouncing. In addition to playful activities during social play, rats also engage in non-playful behaviors including social exploration. The lack of ability to engage in social play with conspecifics is the main indicator of neuropsychiatric disorders like autism. There is strong evidence suggesting that maternal infection during pregnancy is correlated with increased risk of developing autism spectrum disorder (ASD) in the child. To model maternal immune activation, polyinosinic: polycytidylic acid (poly(I:C)) is used to induce inflammatory response in the maternal-placental-fetal axis. The resulting inflammation leads to perturbation of the brain development of pups and consequently may lead to development of the symptoms of autism.

AIM(S): We performed the Social Play Test to study changes in social play behavior of rats in an ASD model induced by prenatal exposure to poly(I:C).

METHOD(S): Pregnant Sprague-Dawley rat dams received a single intraperitoneal injection of poly(I:C) (5 mg/kg) or vehicle at gestational day 15. The 30 – 35 days-old rats were then tested using the Social Play Test.

RESULTS: Rats that were prenatally exposed to poly(I:C) demonstrated a significant decrease in a number of episodes of pinning compared to the vehicle-treat-

ed controls. We also observed significantly diminished time of social exploration in the offspring of poly(I:C) treated females.

CONCLUSIONS: The present study demonstrated that prenatal exposure to poly(I:C) results in social deficits in juvenile rats. Measures of social play behaviours in rats could be useful for quantifying abnormal levels of sociability associated with autism.

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P1.18. PHASE-AMPLITUDE CROSS-FREQUENCY COUPLING IN RAT'S OLFACTORY BULB AFTER INJECTION OF KETAMINE

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INTRODUCTION: The injection of ketamine is an animal model of schizophrenia. It leads to behavioral changes such as hyperlocomotion and accelerated breath and electrophysiological changes such as the appearance of high-frequency oscillations (HFO). Previous studies reported that the amplitude of HFO in the striatum is coupled with a phase of respiratory rhythm. However, recent studies suggest that the olfactory bulb is an important generator of HFO which can impose this activity in ventral striatal areas.

AIM(S): The purpose of this study was to examine the LFP recording from olfactory bulb after injection of ketamine with a novel method of phase-amplitude coupling detection.

METHOD(S): The proposed novel method of PAC detection is based on analysis of time-frequency representation of signals aligned to a given phase in the low-frequency band. Low-frequency wave is obtained with the Matching Pursuit algorithm by selecting waveforms of interest. The time-frequency representation of the signal's energy density is derived from continuous wavelet transform and normalized at each frequency relative to its average value in the baseline period. Next, the representation is thresholded at values obtained from surrogate data. The resulting maps are used to compute comodulograms. The effects presented in the comodulograms are validated with extreme values statistics.

RESULTS: We found statistically significant coupling between the amplitude of high-frequency oscillation (around 150 Hz) and phase of low-frequency oscillation (around 7 Hz) in most of the examined rats. The temporal pattern of PAC shows dependence on injection of ketamine.

CONCLUSIONS: The HFO in olfactory bulb display the property of phase-amplitude coupling with low-frequency oscillation. The additional conclusion is that the proposed novel method is adequate to detect coupling in real LFP data.

P1.19. INCREASED ANXIETY-RELATED BEHAVIOR IN A ZEBRAFISH MODEL OF TUBEROUS SCLEROSIS COMPLEX RECAPITULATED HUMAN SYMPTOMS OF THE DISEASE

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INTRODUCTION: Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder, caused by mutations inactivating genes for proteins hamartin (TSC1) and tuberin (TSC2) and subsequent overactivation of mTORC1. TSC manifests itself by the presence of hamartomas in various organs, although epilepsy is the most influential in mortality. Not only neurological symptoms but also neuropsychiatric manifestations affect TSC patients, which are gathered by the name TSC-associated Neuropsychiatric Disorders (TANDs). The most common disorders are autism spectrum disorder (25-50%), intellectual disability (30-50%), and anxiety (30-60%).

METHOD(S): In our study we used *tsc2*^{vu242} zebrafish mutant line in which a truncating mutation in *tsc2* gene led to lack of Tsc2 protein. We performed three types of behavioral test towards anxiety: Response to Sudden Light Changes, New Environment Exploration, and the Light preference *test.* We also measured cortisol levels in 5 dpf *tsc2*^{vu242} larvae.

RESULTS: In response to the Sudden Light Changes test, *tsc2*$^{vu242/vu242}$ mutant fish exhibited stronger freezing and hyperactivity behavior between dark and light phases compared to *tsc2*$^{+/+}$ fish, which indicates increased anxiety. In the New Environment Exploration test, *tsc2*$^{vu242/vu242}$ mutant fish spent less time exploring the central area of the plate compared to their sibling of other genotypes, choosing safe areas near the edges. Only in the Light Preference test, *tsc2*$^{vu242/242}$ fish presented impaired phototaxis, preferring the dark compartment, while *tsc2*$^{+/+}$ controls show clear positive phototaxis. Elevated cortisol levels in *tsc2*$^{vu242/vu242}$ mutants further confirmed increased anxiety.

CONCLUSIONS: We show that *tsc2*$^{vu242/vu242}$ fish exhibit increased anxiety-related behavior compared with *tsc2*$^{+/+}$ fish, also on the stress hormone level. The Light Preference test points to intellectual disability in the *tsc2*$^{vu242/vu242}$ mutant. These results reflect the human phenotype of TANDs.

P1.20. THE EFFECT OF TRANSCUTANEOUS STIMULATION OF THE VAGUS NERVE ON HIPPOCAMPAL FORMATION THETA RHYTHM IN ANAESTHETIZED RATS

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INTRODUCTION: A number of invasive and noninvasive brain-stimulation techniques are used in clinical neurology. Stimulation of peripheral nerves may affect brain activity through a bottom-up mechanism, for instance, by stimulating cranial nerve nuclei in the brainstem. With specific regard to peripheral methods, studies using vagal nerve stimulation (VNS) have demonstrated that neurostimulation modalities can produce robust therapeutic effects without incurring unsafe consequences on brain function. Just recently, we have demonstrated that direct VNS induced hippocampal (HPC) theta rhythms. The fact that HPC theta rhythm is directly involved in memory processing suggests that VNS can be considered as a useful treatment of patients with Alzheimer's disease.

METHOD(S): Transcutaneous stimulation of the vagus nerve (t-VNS) was performed on the level of external ear in anesthetized rats. Two uninsulated tungsten electrodes (0.1-0.2 kΩ) were used for bipolar VNS through the left lobule of the auricle. Three different points localized on the left lobule of the auricle were tested. The following VNS intensities were applied: 0.2-10 mA. The frequency of VNS was in range (5-60 Hz), pulse duration (1 ms) and train duration (10 s).

RESULTS: Three separate points of localized on the left lobule of the auricle responded with different HPC field responses. The most effective in inducing HPC theta rhythm was the area of entrance of the external auditory canal.

CONCLUSIONS: The data obtained in the project indicated that the entrance of external auditory canal was found to be the most effective in inducing theta rhythm during t-VNS. Further, the effectiveness of t-VNS on HPC theta rhythm is determined by its intensity and local density of vagal endings.

FINANCIAL SUPPORT: These studies were supported by The National Centre of Research and Development (grant no 01.02.00-00-0023/17-001).

P2.1. THE ROLE OF DREBRIN IN THE NEUROMUSCULAR JUNCTION

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INTRODUCTION: Drebrin is an actin-binding protein that regulates cytoskeleton dynamics in different cell types. It plays an important role in shaping dendritic spines and facilitating the stabilization of neurotransmitter receptors at the postsynaptic membrane in the CNS. Its role at the neuromuscular junction (NMJ) in the PNS has not been investigated.

AIM(S): In the present study we aimed to explore the role of Drebrin in this special type of synapse and to study its mechanism of action.

METHOD(S): We used an *in vitro* model of C2C12-derived myotubes in which Drebrin1 expression is silenced with siRNA or its actin-binding function is blocked by a BTP2 inhibitor. To address the role of Drebrin at the postsynaptic machinery, we used both biochemical and immunohistochemical approaches.

RESULTS: We found that Drebrin colocalizes with acetylcholine receptors (AChR) at the surface of myofibers *in vivo* and *in vitro*, and its depletion causes impairments in receptor aggregation and clusters complexity, suggesting a crucial role in the regulation of these processes. We assessed whether drebrin inhibition affects the expression levels and cell surface delivery of AChRs or the microtubule organization underneath AChRs. Our experiments revealed that drebrin depletion in cultured myotubes affects the organization of cortical microtubules, which has been previously shown to be indispensable for incorporation of newly synthesized AChR into the postsynaptic specialization.

CONCLUSIONS: We found that Drebrin is a component of the muscle postsynaptic machinery and it plays an important role in their organization. The mechanism through which Drebrin regulates AChR clustering appears to occur through its interaction with EB3, that leads to the recruitment of microtubules and allows the stabilization of AChRs.

FINANCIAL SUPPORT: This research was supported by the National Science Centre grants: UMO-2018/29/B/NZ3/02675, UMO-2016/21/D/NZ4/03069, and UMO--2018/29/N/NZ3/02682.

P2.2. WHAT PLANNING INTERACTIONS WITH TOOLS CAN TELL US ABOUT BIMANUALITY: AN FMRI STUDY

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INTRODUCTION: Using tools according to their functions requires parallel signal processing in numerous and specialized brain areas. So far, fMRI research on neural substrates underlying interactions with tools was mostly restricted to unimanual, pantomimed tool use.

AIM(S): The scope of this project was to establish whether representations involved in planning bimanual grasps and subsequent usage of real tools can be distinguished from their functionally equivalent unimanual counterparts. Moreover, we addressed a question whether neural activity within the praxis representation network (PRN), responsible for transforming intentions into actions, is modulated by the number of effectors (hands) required to prepare the appropriate action towards a tool (e.g., a functional grasp).

METHOD(S): FMRI contrasts, including repeated-measures ANOVAs and a region-of-interests approach, was adopted. 20 right-handed participants were scanned in two separate sessions involving a leading *vs.* a non-leading/supporting hand. The task was to interact – plan, grasp and execute an action – with bimanual and unimanual tools, and control objects.

RESULTS: The greater engagement of the right superior parietal lobule (SPL) suggests that the primary aspect of bimanuality is coordination. Complex motor-to-mechanical transformations for such synchronized movements take place even before grasp and usage onsets. Although PRN was not modulated by tool manuality, SPL was also involved in initiating interactions with bimanual tools. Finally, as the task progressed from the planning to execution, the processing was more extensive and required more neural resources, peaking at the moment of the functional grasp.

CONCLUSIONS: Even common actions such as grasping bimanual tools have to be preceded by multifaceted neural signal processing. Furthermore, the brain mechanisms underlying these actions are planned well before the actual behavioral performance of a task.

FINANCIAL SUPPORT: Supported by NCN Maestro 2011/02/A/HS6/00174 to GK.

P2.3. THE NUMBER AND MOTOR INNERVATION OF MUSCLE SPINDLES IN THE MEDIAL GASTROCNEMIUS OF MALE AND FEMALE RATS

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INTRODUCTION: There are numerous sex differences concerning male and female skeletal muscles, including muscle mass, the number and diameter of muscle fibers, and number of motor units; however, there is no data concerning the number and density of muscle spindles, which are the most important muscle proprioceptors.

AIM(S): The experiments aimed to determine the number of gamma motoneurons, number and density of muscle spindles, as well as, their morphometric properties for rat medial gastrocnemius.

METHOD(S): The motoneurons were stained with the horseradish peroxidase and neurons exceeding 27.5 μm were accepted as gamma size. Muscle spindles were counted in muscle cut into 5-10 μm sections.

The rat medial gastrocnemius was innervated by a similar number of gamma motoneurons (27.6±3.9 and 28.7±6.6, $p>0.05$) for female and males, respectively.

RESULTS: However, size of gamma motoneurons was higher in males (23.5±2.9 μm) than in females (21.6±2.9 μm) ($p<0.01$). The number of muscle spindles amounted to 13.16±1.25 and 14.0±2.71 for female and males, respectively ($p>0.05$). The diameter of intrafusal muscle fibers was similar in males (5.16±2.43 μm) and in females (5.37±2.27 μm) ($p>0.05$), whereas the number of intrafusal muscle fibers was smaller in females (4.76±1.23) than in males (5.2±1.89, $p<0.05$). The mass of studied muscle was 61% higher in males (1.08 g *vs.* 0.66 g in females); therefore, the muscle mass per one spindle was 77 mg in males and 50 mg in females.

CONCLUSIONS: The number of muscle spindles as well as their motor innervation by gamma motoneurons is similar in male and female rats, but the density of muscle spindles is considerably higher in females.

FINANCIAL SUPPORT: Supported by grant 2018/31/B/NZ7/01028.

P2.4. LONG-LASTING EFFECTS OF TRANS-SPINAL DIRECT CURRENT STIMULATION ON MOTONEURON FIRING PROPERTIES IN RATS

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INTRODUCTION: Trans-spinal direct current stimulation (tsDCS) is a neuromodulative technique used to improve motor functions in neurological disorders and to facilitate sport performance. However, despite the broad application of spinal cord polarization, the physiological mechanisms behind the observed effects remain unclear. We have recently demonstrated that anodal or cathodal tsDCS can alter motoneuron membrane properties and firing characteristics during its application and beyond.

AIM(S): The aim of this study was to determine whether these alterations persist over a longer period of time.

METHOD(S): The study was performed on adult male Wistar rats under general anesthesia. Anodal or cathodal tsDCS (0.1 mA, 15 min) was applied through an electrode located on the lumbar vertebra above the recording site. The intracellular recordings from L4-L5 spinal motoneurons were performed at various periods after

the offset of polarization (up to 3 hours). The animals not subjected to tsDCS formed the control group.

RESULTS: Anodal tsDCS evoked a significant decrease in the voltage threshold, the rheobase, the threshold for rhythmic steady-state firing, as well as, an increase in the steady-state firing frequencies and the slope of the frequency-current relationship. Some of these modulatory effects were observed up to 60 minutes after the offset of polarization. Cathodal tsDCS induced only modest changes in motoneuron threshold properties, which could be observed no longer than 30 minutes after the end of polarization.

CONCLUSIONS: This study for the first time provides the direct evidence that tsDCS evokes long-term alterations in the threshold and rhythmic firing properties of spinal motoneurons. Modulatory effects of anodal polarization are stronger and last longer than those of cathodal tsDCS. We suppose that both autonomous cell mechanisms and synaptic effects contribute to the occurrence and long-term persistence of the indicated changes in motoneuron properties.

FINANCIAL SUPPORT: Supported by the National Science Center grant No 2017/25/B/NZ7/00373.

P2.5. THE INFLUENCE OF ENDURANCE, STRENGTH, AND VIBRATION TRAINING ON SAG IN UNFUSED TETANIC CONTRACTIONS OF FAST MOTOR UNITS

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INTRODUCTION: Unfused tetanic contractions of fast motor units exhibit a transitional decline in force following the initial extra-efficient force development, known as sag. Sag is sensitive to changing energy demands and the fuels metabolized to meet these demands.

AIM(S): Since different training modes have varying effects on cellular energy systems in muscles, we aimed to determine how endurance, strength, and vibration training would affect sag in fast motor units of rat medial gastrocnemius.

METHOD(S): Separate control groups were used for each training mode, with activity limited to normal cage movements. Endurance training (ET): 2, 4, or 8 weeks of treadmill training with weekly progressions in duration and speed. Strength training (ST): 5 weeks of voluntary progressive weight-lifting. Vibration training (VT): 3 or 6 months of whole-body vibration training at 50 Hz (4 × 30 s with 60 s rest periods). ET, ST, and VT groups were trained 5 days per week. Following training, functionally isolated fast motor units (divided into fast fatigable (FF) and fast fatigue-resistant (FR)) were investigated, and profiles of their unfused tetanic contractions at 40 Hz with sag were analyzed.

RESULTS: The 40 Hz contractions of trained groups were less fused than those of untrained groups; this effect was attributable to shorter twitch time parameters in trained animals. Accordingly, numerous differences appeared in the sag profiles. However, when limiting the comparisons to motor units with comparable levels of fusion (fusion index 0.2 – 0.8), few differences were observed. With this constraint 1) ET had no effect on sag profiles, 2) the force decreased after the initial peak was delayed in FF of ST rats, and 3) VT resulted in a shorter duration of sag in FF and a smaller force decrease after the initial peak in FR.

CONCLUSIONS: We conclude that while different training modes have differing effects on sag, these effects are primarily due to altered twitch time parameters.

P2.6. HYPOTHERMIA INDUCED CHANGES IN CONTRACTILE PROPERTIES OF MOTOR UNITS IN RAT MEDIAL GASTROCNEMIUS

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INTRODUCTION: Experiments concerning the influence of temperature on mammalian muscles have reported that contractile properties and metabolism are sensitive to the temperature. However, in the literature, there is no information concerning consequences of reduced temperature for the motor unit (MU) contractile properties. Based on available data, we expected predominantly a slowdown in twitch time parameters of three types of MUs.

AIM(S): The main goal of this study was to determine how hypothermia modifies MUs contractile properties.

METHOD(S): Experiments were performed on adult Wistar anesthetized rats. MUs were activated by electrical stimulation of single axons isolated from ventral roots of spinal nerves. Two groups of animals were tested: 1) control (at physiological temperature 37±1°C) and 2) hypothermia (at 25±1°C).

RESULTS: We observed that hypothermia increased delay of twitch in FF and FR but not in S MUs. Furthermore, the twitch time was considerably prolonged in FF and FR MUs in contrast to S ones. The half relaxation time (HRT) was significantly slower in all types of MUs in the hypothermia group. The twitch force was lower in FF MUs, while in FR and S MUS we observed moderate differences between hypothermia and control groups. Finally, tetanus force was also significantly lower in FF,

but we have not found significant differences in FR and S MUs. The twitch-to-tetanus ratio was considerably higher in hypothermia, indicating a reduced possibility of force regulation by changes in motoneuronal firing.

CONCLUSIONS: Hypothermia dramatically reduced motor control processes and force regulation of MUs in skeletal muscles. The results indicated that FF and FR MUs are more sensitive to the influence of low temperature than S ones.

P2.7. NEURAL BASES OF ACTIONS INVOLVING COMPLEX MOTOR-TO-MECHANICAL TRANSFORMATIONS

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INTRODUCTION: Each human action involving tools requires transformations of motor responses of hands/fingers (or different effectors) into mechanical actions of a tool. Whereas tools requiring a relatively low level of motor-to-mechanical transformations were extensively studied, little is known about the neural underpinnings of complex tool use, which involves compound motor-to-mechanical transformations.

AIM(S): The aim of this study was to investigate the neural bases and mechanisms of functional interactions with tools that are characterized by a high level of motor-to-mechanical transformations.

METHOD(S): Functional magnetic resonance imaging (fMRI) was utilized in 20 right-handed participants when they prepared and performed multi-phase purposeful actions with real complex tools, with their right and left hands. Specifically, neural activity was investigated during the planning and execution of functional grasp and subsequent usage of complex tools, as compared to actions involving simple tools and control, non-tool objects.

RESULTS: Although specific neural engagement for complex tools, as compared to non-tools, was observed in all phases and concerned nodes of the left-lateralized Praxis Representation Network (PRN), complex tools *vs.* simple tools, were processed differently at the grasping and tool-use programming stage. In this phase, the grasping action directed at complex tools involved more the intraparietal sulcus and the nearby subdivisions of the superior parietal lobule. Interestingly, more thorough analyses demonstrated that such translations from motor to mechanical codes also engaged the rostral inferior parietal lobule.

CONCLUSIONS: These outcomes point to the prospective character of grasp coding, a process associated with the activity of the intraparietal sulcus, and its cooperation with other hand-centered and tool-centered mechanisms during performance of motor-to-mechanical transformations.

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P2.8. THE ROLE OF AMOT AND YAP1 IN THE REGULATION OF DENDRITIC TREE MORPHOGENESIS AND CEREBELLAR FUNCTIONS

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INTRODUCTION: The Amot-Yap1 complex plays a major role in the regulation of cell contact inhibition, cellular polarity and growth in many cell types. However, the function of the Amot and Hippo pathway transcription co-activator Yap1 in the CNS remains unclear. Recent studies have demonstrated that, in mature hippocampal neurons, Amot localizes to dendritic spines where it associates with synaptic protein and regulates actin cytoskeleton. However, its function during neuronal development has not been studied.

METHOD(S): Cultured primary neurons were used for RNAi experiments. For *in vivo* functional analysis, we used Amot and Yap1 conditional KO mice. For deletion in single sparse neurons, mice were injected with low doses of AAV-CRE. For behavioral analysis, we used rotarod, catwalk, and foot fault tests.

RESULTS: We demonstrate that Amot is a critical mediator of dendritogenesis in cultured hippocampal cells and Purkinje cells in the brain. Amot function in developing neurons depends on interactions with Yap1, which is also indispensable for dendrite growth and arborization *in vitro*. Conditional deletion of Amot or Yap1 in neurons leads to decreased Purkinje cell dendritic tree complexity, abnormal cerebellar morphology, and impaired motor coordination. The ability of Amot and Yap1 to regulate dendritic growth depends on regulation of S6 kinase activity and phosphorylation of S6 ribosomal protein. Hence, we suggest that Amot and Yap1 control dendritic tree morphogenesis through a cross-talk with the PI3K/mTOR pathway, a known regulator of dendritogenesis.

CONCLUSIONS: We identify a novel role for the scaffold protein Amot and the Hippo pathway transcription co-activator Yap1 in dendritic morphogenesis.

FINANCIAL SUPPORT: This research was supported by National Science Center grants 2012/05/E/NZ3/00487 and 2015/19/N/NZ3/02346.

P2.9. EFFECT OF A 4-WEEK MENTAL TRAINING ON DECREASE IN GRASPING FORCE IN YOUNG HEALTHY PEOPLE

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INTRODUCTION: Not only muscle contraction, but also muscle relaxation plays an important role for performance of voluntary movements. Most of the studies have focused on muscle contraction rather than on their relaxation. Research on motor ability has established clearly that mental practice leads to improved execution of movement.

AIM(S): Assessment of the effect of a four-week mental training on cortical activity related to decrease in grasping force in healthy, young people.

METHOD(S): 15 healthy subjects (8 men and 7 women) between 23 to 33 years voluntarily participated in the study. Mental training (MT) lasted 4 weeks with 3 training sessions per week and cortical activity using 128-channel EEG system was recorded in all subjects during two measurement sessions (before and after the MT). During sessions subjects performed: 3x maximal isometric voluntary contraction (MVIC) in grasp function, 40 repetitions at submaximal level of force (20% of MVIC) during the same task and 2 × MVIC. The amplitudes of motor related cortical potentials (MRCP) of the EEG signal were analyzed in the BESA software (BESA GmbH, Germany) for electrodes placed in the areas associated with the planning and execution of movements on averaged files from 20% MVIC part of the protocol and triggered around (from -3 s to 1 s) decrease in grasping force. To compare the MRCP values before and after MT, the Wilcoxon signed-rank test was performed in SPSS (IBM SPSS 22.0, USA) with the level of significant that was set at $P \leq 0.05$.

RESULTS: Analysis of the MRCPs did show significant differences between relaxation amplitudes before and after the MT.

CONCLUSIONS: Muscle relaxation is accompanied by activation of the premotor cortex (PM), primary motor (M1), primary and secondary somatosensory areas (S1, S2). The level of the cortical activity associated with relaxation of the muscles during precise grasp movement performed by right upper limb was higher after MT especially in the S1, S2 and PM areas compared to M1.

P2.10. EFFECT OF MOTOR IMAGERY TRAINING OF REACHING-TO-GRASP ON CORTICAL ACTIVITY RELATED TO MOTOR IMAGERY AND MOTOR EXECUTION OF GRASPING BY DOMINANT HAND IN HEALTHY PEOPLE

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INTRODUCTION: Motor imagery can be defined as a dynamic state during which a subject mentally simulates a given action. Conscious motor imagery and unconscious phase of motor planning potentially share common mechanisms. Voluntary movements, such as goal-directed reaching for an object, are those that are governed by motor programs and require planning.

AIMS(S): The aim was to assess the effect of 4-weeks of motor imagery training of reaching-to-grasp on cortical activity related to motor imagery of grasping (a book) and motor execution of grasping by dominant hand in healthy, young subjects.

METHOD(S): 11 volunteers between 23 and 33 years participated in this study. There were: 2 measurement sessions (before motor imagery training (MIT) and after MIT) and 4-weeks of kinesthetic MIT of reaching for a book (3 × a week) in the study's protocol. During sessions 128-EEG signal during motor imagery of grasping a book (MIG) and motor execution of grasping (MEG) by dominant hand was recorded. The analysis of EEG was made in BESA software and amplitudes of event related potential (ERP-related to motor imagery) and motor related cortical potential (MRCP-related to motor execution) from regions of motor programming and execution were obtained. The statistical analysis was made in SPSS software and for main comparison multivariate analysis of variance with repeated measures was used to assess effect between sessions, tasks and electrodes' location with $P \leq 0.05$.

RESULTS: The analysis has shown significant effect of electrodes' location and tasks on EEG amplitude. There were no significant effect of session and interaction between factors.

CONCLUSIONS: Mental training is important method to improve movements' execution but its neural mechanisms are still unclear. Our study has shown that MIG and MEG share similar amplitude patterns in areas related to motor programming. We have observed a similar tendency to increase ERP and MRCP amplitudes as an effect of MIT.

P3.1. EVALUATION OF SPATIAL GENERALIZATION IN HOUSE CRICKET (*ACHETA DOMESTICUS*)

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INTRODUCTION: Navigation in dynamic environments is a task of vital importance in the survival and reproduction of animals. However, due to the dynamism of processes occurring in natural environments, featural consistency of objects throughout the time could not suffice to identify them. One of such is an arrangement of objects in space. For example, while trees tend to drop their leaves, they relatively rarely change their place. Despite the recent breakthrough in the understanding of mammalian navigation, the question of how insects, which have a radically different nervous system architecture, find their way in equally complex environments remains open.

AIM(S): In the present study, the authors attempted to test whether the house cricket (*Acheta domesticus*) is able to navigate by the general shape of the arena and locate its center.

METHOD(S): The experimental setup consisted of a set of heated areas of different shapes (circular, rectangular, triangular, quadrilateral) with a cool spot located centrally (Tennessee Williams paradigm). Arenas were devoid of visual, tactile, and olfactory cues; all the tests were conducted in an acoustically isolated environment. During the consecutive trials, crickets were released on the arena and tracked with object-tracking software. Thereafter, acquired tracks were analyzed and the time spent on cool spot summated.

RESULTS: In all tested arenas except the quadrilateral one, in subsequent trials (median on time spent in the group of all tested insects 10±5% in the first trial *vs.* 40±5% in the tenth trail) insects tend to spend more time on the cold spot. However, learning curves varied for different shapes.

CONCLUSIONS: Obtained results point that insects seem to be able to rely on generalized information of environmental geometry, yet further, in-depth analysis is required to explain possible mechanisms of such ability as well as the differences were observed for the navigation in different shapes.

P3.2. KETOGENIC DIET AND SEXUAL MOTIVATION IN MALE RATS

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INTRODUCTION: The ketogenic diet (KD) is a very-low-carbohydrate, high-fat and adequate protein nutritional approach that induces metabolic shift to the use of ketone bodies instead of glucose as a main energy source. For decades, the KD has been employed to manage drug-resistant epilepsy, but recently it is increasingly considered as an alternative or add-on therapy in many other disorders. The positive effect of the KD on social behavior has been recently reported in rodent models of autism spectrum disorder (ASD) and wild type early adult male rats.

AIM(S): Given, however, the influence of ketone bodies on many humoral parameters including testosterone, we decided to look closer at the sexual interactions of male rats subjected to the KD.

METHOD(S): In our study, we examined behavior of males in response to female presence with concomitant analysis of ultrasonic vocalizations (USV) during sexual interactions.

RESULTS: Percent of males starting to copulation significantly decreased during the second sexual session, which suggests lower sexual motivation in male rats on the KD.

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P3.3. FISH AS ANIMAL MODELS IN BIOMEDICAL RESEARCH

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INTRODUCTION: In biomedical research, there is a constant lookout for animal models that can help in the search for new drugs and therapies. The most commonly used are laboratory mice and rats, but their use is under pressure of the need to reduce used animals. Many experiments can be performed on simpler vertebrates such as fish, but even here the choice of species is crucial.

METHOD(S): I used a comparison between the same types of experiments conducted on fish and other groups of animals based on published data.

RESULTS: Regarding complex cognitive mechanisms, there is experimental evidence for diverse processes such as cognitive maps, transitive inference, complex social learning rules, referential gestures, generalization, or mirror recognition in fish. Recent research on vertebrate brains has also identified amazingly con-

served structures with respect to a so-called social decision-making network, which consists of the 'social behaviour network' and the 'basal forebrain reward system'.

CONCLUSIONS: Given these similarities, fish seem to offer vast opportunities for testing general principles concerning social behavior and underlying cognitive mechanisms and processes. There is also a need to create special equipment to study more sophisticated behavioral tasks in fish that could be compared with that of rats and mice. Additionally, behavioral tests combined with the analysis of the brain structure will allow us to understand the differences in processing information concerning social and environmental behaviors.

P3.4. SOCIAL INTERACTIONS AND ULTRASONIC VOCALISATION IN SEROTONIN TRANSPORTER KNOCKOUT RATS

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INTRODUCTION: The serotonergic system has been implicated in several CNS activities, including social behaviour. The serotonin reuptake transporter (SERT) plays a key role in the regulation of serotonergic system functioning. Therefore, rats lacking SERT ($SERT^{-/-}$) represent a valuable model to study the consequences of constitutive increases in serotonin concentrations. In adult laboratory rats, two main types of ultrasonic vocalisations (USVs) have been described: the low (22-kHz) and high (50-kHz) frequency calls. The low, termed an "alarm" vocalization, has been associated with negative social experiences. The high may be detected in appetitive contexts, including social interactions.

AIM(S): The goal of the current study was to examine male $SERT^{-/-}$ and $SERT^{+/+}$ rats in the social interaction test to investigate genotype differences in social behaviour and communication.

METHOD(S): Two unfamiliar rats of matched body weight were placed in the open field arena, and their behaviour was recorded. Durations of the following behaviours were scored: social contact behaviour (including sniffing, anogenital sniffing, social grooming, and mounting/climbing) and following the partner. Additionally, USVs were measured during the social interaction tests.

RESULTS: We report that $SERT^{-/-}$ rats spent significantly less time on social contact but demonstrated more of partner following behaviour as compared to $SERT^{+/+}$ rats. There were no effects of genotype on the number of 22-kHz and 50-kHz USVs emitted during social interaction. However, serotonin transporter deletion affected the distribution of sound categories in that $SERT^{-/-}$ rats demonstrated a decrease in the percentage of complex calls and an increase in the percentage of trill and step calls.

CONCLUSIONS: The current study further supports the role of serotonin in the regulation of social and communicative behaviour.

FINANCIAL SUPPORT: This study was supported by the grant ERA-NET Neuron II JTC 2015 Respond.

P3.5. PRESENCE OF A COMPANION ALLEVIATES FEAR RESPONSE BUT DOES NOT EQUAL FEAR EXTINCTION

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INTRODUCTION: Social support during exposure-based psychotherapy has been suggested to have an important influence on the course of exposure treatment, however some clinical trials show that individual therapy may be more effective than group therapy. The mechanisms of social influence on fear extinction remain unknown.

METHOD(S): To study neuronal correlates of social buffering in fear extinction, we have developed a rat model. In our model, rats showed a significant lowering of fear response during fear extinction when exposed to fear-associated stimuli with a companion. The buffering magnitude depended on familiarity and physical similarity of the tested animals but not on their emotional status; the fear-conditioned partners were as effective as naïve ones. However, the effect was transient and disappeared when rats were tested individually the next day. To test whether social buffering shares neuronal mechanisms with fear extinction, we measured activation of fear regulating neuronal circuits. Lower fear response during exposure with a partner was associated with lower activation of the infralimbic (IL), prelimbic (PL), and anterior cingulate (ACC) cortices. However, although optogenetic blocking of the IL increased fear response in rats tested separately, it left the social buffering effect intact.

RESULTS: Analyzing inputs to the cortex from the ventral hippocampus (vHIPP) and basolateral amygdala (BL), we found significantly more vHIPP innervated neurons activated in the PL but not IL or ACC of the socially buffered rats.

CONCLUSIONS: The results show that fear memory suppression by the presence of a companion is transient and relies, at least partially, on different neuronal circuits than fear extinction.

P3.6. THE INFLUENCE OF FATTY-ACID AMIDE HYDROLASE INHIBITORS ON MEMORY-RELATED BEHAVIORS PROVOKED BY CHOLINERGIC RECEPTOR LIGANDS IN MICE

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INTRODUCTION: The endocannabinoid system (ECS) is composed of cannabinoid (CB: CB1 and CB2) receptors and endocannabinoids, which are degraded by fatty acid hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Thus, the function of the ECS might be modulated in a direct way, through CB receptor ligands or indirectly by FAAH and MAGL inhibitors. The ECS system is involved in many physiological functions, also through interaction with many systems. The cholinergic system plays a crucial role in memory processes. A connection with the ECS, especially CB and cholinergic receptor ligands, is supported by a large body of research. However, the influence of the ECS through an indirect manner in the context of cognitive processes remains poorly understood.

AIM(S): The aim of the study was to evaluate the indirect influence of ECS, using of FAAH (URB 597) and MAGL (JZL 184) inhibitors, on memory related effects provoked by cholinergic receptor ligands, a cholinergic receptor agonist, nicotine, and a cholinergic receptor antagonist, scopolamine, in mice.

METHOD(S): We assessed different memory stages using the passive avoidance (PA) test. A deficit in PA performance was expressed as the difference between retention and training latencies and is taken as an index of latency (IL).

RESULTS: Co-administration of non-effective dose of JZL 184 (4 mg/kg), but not URB 597 (0.1 mg/kg), with a non-effective dose of nicotine (0.05 mg/kg) enhanced both acquisition and consolidation of memory in the PA test in mice. An acute injection of JZL 184 (4 mg/kg) attenuated pro-cognitive effects induced by effective dose of nicotine (0.1 mg/kg). In turn, co-administration of URB (0.1 mg), but not JZL 184 (4 mg/kg), with scopolamine (1 mg/kg) attenuated the scopolamine-induced memory impairment in the PA test in mice.

CONCLUSIONS: The present findings clearly indicate that the ECS, through an indirect manner, modulates memory processes, especially those in which cholinergic pathways are implicated.

P3.7. AMOTL1: A NOVEL SYNAPTIC PROTEIN IMPORTANT FOR MICE SOCIAL BEHAVIOR AND BRAIN ORGANIZATION

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INTRODUCTION: The angiomotin family comprises of three scaffold proteins – Amot, Amotl1, and Amotl2 – that have been implicated in the regulation of cell polarity, migration, and proliferation. Recent *in vitro* studies have reported that Amot localizes to the synapses in mature neurons and regulates dendritic spine maturation.

AIM(S): We have found that Amot, together with Yap1, the Hippo pathway transcription co-activator, are critical for proper dendritic arborization and mice locomotor coordination. However, to date the function of the two other Angiomotins, Amotl1 and Amotl2, in neurons has not been investigated.

METHOD(S): To study Amotl1 function in the mouse brain, we generated systemic and neuron-specific knock-out (KO) mice. To assess general locomotion, we performed an open field test. Amotl1 KO mice sociability was evaluated with the three-chamber task, automatic Eco-Hab approach, and nesting test. To record the animal's anxiety response, we used the marble burying test.

RESULTS: In the present study, we show that Amotl1 localizes to the synaptic compartments in neurons. Deletion of Amotl1 leads to hyperlocomotion, decreased anxiety-like behavior, and alteration in mice sociability. Amotl1 ablation causes an increase in volume of lateral ventricles in the mouse brain by 50%. These features have been previously observed in animal models of various psychiatric disorders, such as schizophrenia or autism. Interestingly, mass spectrometry analysis of neuron-specific interactors demonstrated that Amotl1 binds to FMR1 and FXR1, mutations of which cause Fragile X syndrome.

CONCLUSIONS: We identified a novel synaptic protein, Amotl1, the deletion of which causes behavioral deficits and that it could be a potential molecular target for the development of new therapeutics for neurological disorders.

FINANCIAL SUPPORT: This research was supported by National Science Center (NCN) grants: UMO-2018/29/B/NZ3/02675, UMO-2018/29/N/NZ3/02682.

P3.8. TIME-RELATED DISCRIMINATORY STIMULUS FOLLOWING LSD ADMINISTRATION IN RATS

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INTRODUCTION: Drug discrimination is an important technique in behavioral pharmacology since the last 40 years. LSD affects multiple receptor subtypes of the serotonergic system and is one of the most powerful agents producing psychedelic effects.

METHOD(S): Male Sprague Dawley rats were trained in two lever operant conditioning chambers using the FR10 schedule of reinforcement to discriminate 0.08 mg/kg LSD from vehicle, 15 min following administration. The experiment was conducted 5 days/week during the light phase of the light/dark cycle.

AIM(S): The aim was to examine the time after which the animals were able to discriminate two treatment conditions. Animals were tested 5, 15, 30, and 90 min after 0.08 mg/kg of LSD administration.

RESULTS: All animals tested 15 min following LSD administration choose the LSD lever. In the test carried out after 5 min following injection, 70% of rats choose the LSD lever. The subjects tested after 30 min displayed similar discrimination as tested following 15 min. However, after 90 min following LSD administration, the rats choose the vehicle lever.

CONCLUSIONS: The present data suggest that the cue produced by LSD in the drug discrimination test could be detected shortly after its administration.

P3.9. A TEST FOR ASSESSING PROSOCIAL BEHAVIOR IN MICE

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INTRODUCTION: Prosocial behaviors may be broadly defined as actions that benefit others. While these behaviors are intuitively simple, the underlying mechanism driving prosocial actions are only partly understood, and the methods to observe them under laboratory conditions remain limited.

AIM(S): Here, we describe a novel task that assesses prosocial choices in mice and tests the frequency of prosocial behaviors in male and female C57BL/6 animals.

METHOD(S): The test measures prosocial behavior towards a familiar conspecific. The tested mouse (actor) is placed in the starting compartment of the cage, from which it may enter two feeding compartments. The stimulus mouse (partner) is placed in the compartment adjacent to both actor's feeding compartments. The wall between actor's and partner's compartments is transparent and perforated. Entering the compartment that was designated as "prosocial" by the actor results in the reward delivery to both actor and partner. Entering the "asocial" compartment results in a reward only for the actor. Pilot experiments were also carried out on an automated version of the test using a Skinner box-based setup.

RESULTS: We found that, on average, male mice had no significant preference for the prosocial choice (n=10, 47.5% ± 9.2% without a partner *vs.* 47.8±4.9% with a partner). In the case of females, we initially observed a trend towards increased preference of the prosocial decision, but the result did not reach significance when all experiments were pooled (n=14, 46.9±8.0% without a partner *vs.* 50.8±8.0% with a partner).

CONCLUSIONS: Further experiments are required to conclude whether C57BL/6 mice show prosocial behavior towards conspecific cagemates. The results so far suggest that prosocial behavior may possibly be exhibited by females, which is consistent with observations in wild mice.

P4.1. UV LIGHT DETECTION BY THE RAT OLIVARY PRETECTAL NUCLEUS

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INTRODUCTION: The retina is specialized to detect and process light information by three types of cells: rods, cones, and melanopsin cells. In rodents, S-cones are maximally sensitive to 359 nm light, thus enabling them to see and use light in the UV range. The olivary pretectal nucleus (OPN) receives strong retinal innervation and is responsible for pupillary light reflex (PLR), which depends mostly on melanopsin cell activity.

AIM(S): The aim of this study was to verify whether neurons within the OPN respond to monochromatic light stimuli in the UV range.

METHOD(S): The experiments were carried out on 4 adult Long Evans rats under urethane anesthesia subjected to *in vivo* multi-unit extracellular recordings. The contralateral eye was stimulated by high-irradiance white light pulses and monochromatic light in the 340–490 nm range (3 s, 10 nm interval).

RESULTS: The stable activity of 43 neurons was recorded within the OPN borders and majority of them (84%) were classified as sensitive to white and monochromatic light stimuli in the UV range. OPN neurons

mostly responded in a sustained manner (tonic excitation), even to short wavelength light. Moreover, they were the most sensitive to 380 nm wavelength of light from the UV range.

CONCLUSIONS: The current study shows that light in the UV range widely actives OPN cells. In contrast to retinal studies, the majority of OPN neurons demonstrated sustainability and preferability towards 380 nm wavelength of light. These results suggest that S-cones may contribute to non-image forming functions, such as PLR.

FINANCIAL SUPPORT: Supported by: 2013/08/W/N23/00700.

P4.2. CHEMOGENETIC INHIBITION OF SOMATOSTATIN INTERNEURONS ALTERS PLASTICITY INDUCED BY SENSORY DEPRIVATION IN THE BARREL CORTEX OF MICE

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INTRODUCTION: Among cortical inhibitory cells, activity of somatostatin interneurons (SST INTs) has been recently proposed as a key player in the formation of neuroplastic changes. Sensory deprivation causes changes in inhibitory systems that lead to disinhibition of the spared barrel, allowing for spreading of its functional representation. Because of their unique pattern of connectivity, we hypothesize that layer IV SST INTs strongly modulate disinhibition of the spared barrel, supporting the sensory deprivation-induced plastic change formation.

AIM(S): Using a chemogenetic approach, we aimed to study a direct role of layer IV SST INTs activity in plastic change formation induced by sensory deprivation in mice barrel cortex.

METHOD(S): SST-Cre mice were unilaterally injected with Cre-dependent AAV2 vectors expressing inhibitory DREADDs into a single barrel of row C. Two weeks later, mice underwent a sensory deprivation paradigm, in which all whiskers but one, C3, on one side of the snout were plucked for a week. During deprivation, the activity of SST INTs was blocked by DREADDs activation with its agonist, CNO, continuously administered *via* Alzet® Osmotic Pumps. To visualize plastic change, [14C]-2-deoxyglucose brain mapping was performed. The area of functional representation of the spared whisker and contralateral one was compared.

RESULTS: We found that SST INTs inhibition in the spared barrel did not influence the area of activation of the spared whisker compared to transduced animals with saline administration instead of CNO. However, SST INTs blockade in the deprived barrel, adjacent to the spared one, led to a dramatic decrease in functional plasticity of spared whisker representation.

CONCLUSIONS: Our results indicate that layer IV SST INTs activity in deprived, but not spared barrel, is essential in sensory deprivation-induced plastic change formation in the barrel cortex of mice.

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P4.3. KCNB1 PLAYS A ROLE IN THE DEVELOPMENT AND FUNCTION OF THE EAR IN ZEBRAFISH

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INTRODUCTION: Voltage-gated potassium channels (Kv) are selective transmembrane proteins that allow transport of K^+ across the plasma membrane, where they play an important role in establishing the resting potential. They are involved in a variety of biological processes crucial for proper functioning of the cells. In this study we are focused on zebrafish Kcnb1 (Kv2.1), a member of the electrically active Kv2 subfamily of voltage-gated potassium channels. Previously, it has been shown that this protein is expressed in mammalian, *Xenopus laevis*, and zebrafish inner ear. Based on this, Kv2.1 could be important for development of this organ, where it may be required for proper hearing and spatial orientation. These functions include activity of the mechanosensory cells, which develop a long kinocilium used to export Ca^{2+} required to form and tether the "hearing stones" – the otoliths (in fish) or otoconia (in mammals).

AIM(S): The aim of this study is to investigate the role of Kcnb1 during ear development using zebrafish as a model.

METHOD(S): We used morphometric and behavioral analyses to study development of the ear and check hearing and spatial orientation in Kcnb1 mutants and morphants. Using qRT-PCR we checked level of expression of ear marker genes. Immunohistochemistry was used to stain cilia of the hair cells.

RESULTS: Ears of developing zebrafish *kcnb1*$^{-/-}$ are affected resulting in abnormal morphology and function. Mutants show a significant reduction in size of ears and otoliths as compared to wild-types. We found changes in the morphology of the hair cells in the ear of *kcnb1*$^{-/-}$ with changes in orientation of kinocilia. Upregulation and downregulation of some of ear marker genes has been confirmed. Behavioral tests showed defects in hearing and balance in *kcnb1*$^{-/-}$ and knockdowns.

CONCLUSIONS: Our results support a hypothesis that Kcnb1 is important during development and function of the zebrafish ear.

P4.4. UV LIGHT DETECTION BY THE RAT DORSAL LATERAL GENICULATE NUCLEUS

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INTRODUCTION: The dorsal lateral geniculate nucleus (dLGN) of the rat thalamus is a relay structure that receives light signals from three types of retinal photoreceptors: rods, cones, and melanopsin cells. From three types of cones, only S-cones allow rodents to see light in the ultraviolet range and further use it to image and non-image forming visual functions, including classic vision during the night.

AIM(S): S-cones are maximally sensitive to UV light peaking at 359 nm; however, an unanswered question is whether dLGN cells share a similar wavelength characteristic.

METHOD(S): Thus, we performed *in vivo* extracellular multi-electrode recordings in 5 fully pigmented adult male Long Evans rats under isoflurane anaesthesia combined with monochromatic light stimulations. Animals were dark adapted for 30 min and then different light stimuli in the UV range (3 s, 490-340 nm, 10 nm interval) were presented to the rat's contralateral eye, while recording neuronal activity of LGN cells.

RESULTS: High-irradiance light in the UV range elicited responses in 42% (n=38) of light-sensitive neurons within the dLGN. Recorded neurons were the most sensitive to 380 nm wavelength of light and responded mostly in a transient manner in terms of the shape of evoked photoresponse. Moreover, they did not show any spatial distributions across the LGN.

CONCLUSIONS: Our study confirms that light in the UV range activates the dLGN and can play an important role in image forming functions. In contrast to previous retinal studies, we found out that dLGN cells are most effectively excited by 380 nm.

FINANCIAL SUPPORT: Supported by: 2013/08/W/N23/00700.

P4.5. EXPERIENCE-DEPENDENT ACQUISITION OF VISUOMOTOR BEHAVIORAL REPRESENTATION IN PRIMARY VISUAL CORTEX

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INTRODUCTION: The role of primary sensory cortical areas in perception and behavior remains unclear. Moreover, the functional plasticity of these circuits during task acquisition is largely unknown.

AIM(S): Here, we developed a visual learning task in awake, head-fixed mice in which animals learn to associate a small drifting grating stimulus with an aversive air puff to the cornea, driving the establishment of a conditioned blink response.

METHOD(S): We previously showed that both task acquisition and performance require intact primary visual cortex (V1). Pairing this approach with 2-photon calcium imaging of identified neuronal subpopulations in V1, we monitored cellular activity across two weeks of learning.

RESULTS: Our results show that the population activity of excitatory neurons in both layer 2/3 and 5 reliably encodes the presence of a sensory stimulus throughout training, but acquires the ability to accurately represent motor output over several days. Analysis of individual neurons demonstrates that cells not encoding behavior significantly lose their visual responses during learning, producing an overall enhancement of the population-level representation. We find similar results for GABAergic interneurons expressing parvalbumin and vasoactive intestinal peptide. However, somatostatin-expressing interneurons fail to encode behavior at any point in training, suggesting that cell type-specific mechanisms promote plasticity in V1 circuits associated with learning.

CONCLUSIONS: In conclusion, our data suggest that visual experience produces a functional reorganization of both excitatory and inhibitory networks that facilitates efficient performance in visuomotor behavior.

P5.1. WHOLE-BRAIN MAPPING OF NEUROPLASTICITY IN DIFFERENT EXPERIMENTAL PARADIGMS IN MICE – A COMPUTATIONAL PERSPECTIVE

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INTRODUCTION: Imaging of entire brains at cellular resolution, enabled by light-sheet fluorescence microscopy (LSFM) and optical tissue clearing, offers insights into neural activity at a high magnification while preserving the brain-wide context.

AIM(S): We propose a set of open-source computational tools that address three fundamental challenges associated with the analysis of LSFM images of entire rodent brains, namely: management of voluminous imaging data, alignment to a reference atlas, and object detection and localization.

METHOD(S): The data for each brain, such as multichannel acquisitions and spatial information, are compressed and stored in an HDF5-based container as a pyramid of resolutions to facilitate and standardize data access and manipulation. Unlike most other alignment approaches, our pipeline is not only guided by standard similarity metrics such as mutual information, but also utilizes Deep Convolutional Neural Networks to generate label maps corresponding to specific brain structures such as main white matter tracts or dentate gyrus. This step significantly increases the accuracy and robustness of the registration procedure. The c-Fos-positive nuclei are identified and quantified with the help of another neural network trained on synthetic data, generated to simulate the original nuclei which eliminated the laborious process of manual image annotation.

The software was applied to investigate c-Fos-mediated neuroplasticity in iDISCO-cleared brains in experimental paradigms of appetitive and aversive learning and alcohol addiction.

RESULTS: Voxel-wise statistical analysis revealed brain areas involved in the neuroplasticity of alcohol addiction and appetitive or aversive learning in mice.

CONCLUSIONS: We demonstrate the ability of our software to combine efficient data management, accurate atlas alignment, and object detection to facilitate LSFM analyses.

FINANCIAL SUPPORT: ERA-NET NEURON/17/2017 grant from NCBR, G2631 grant from NCN.

P5.2. EXPLORATORY AND CLASSICAL ANOVA ANALYSIS OF RESPONSE-LOCKED EVENT-RELATED POTENTIALS

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INTRODUCTION: In the classical approach, we assume that when designing the experiment in the way described in the literature, we can observe specific components of the event-related potential (ERP) induced in specific areas of the brain with specific latencies. Using these standard methods of data analysis, i.e., looking for activity changes only in components commonly known from the literature, there is a risk of not noticing new, interesting effects.

METHOD(S): In order to check if the data-driven approach gives the opportunity to verify the classical approach and whether it allows to better match the analysis, we compared it with the classical analysis, using data from emotional experiments. We investigated the electrophysiological correlates of execution of an ambiguous task under the influence of emotionality of words stored in working memory.

RESULTS: The analysis of variance (ANOVA) classical analysis of ERP was compared with an exploratory approach using GFP (Global Field Power), calculated as spatial standard deviation. Analysis of the GFP curve was used to determine the time periods in which we performed a 4-factor ANOVA with repeated measures.

CONCLUSIONS: In the present case, we were able to find significant effects related to the valence and origin consistent with classical analysis while maintaining control of the statistical significance. Phenomena were shifted in the time domain and with a tilted pattern in the spatial distribution.

P5.3. MICROELECTRONIC SYSTEM FOR LOW-ARTIFACT ELECTRICAL STIMULATION AND RECORDING OF BRAIN ACTIVITY AT UP TO 512 ELECTRODES

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INTRODUCTION: We present a novel microelectronic system for *in vivo* stimulation and recording of neuronal activity. The system is intended for use with multielectrode silicon probes and is based on a dedicated 64-channel CMOS chip. It can generate complex sequences of microstimulation pulses and simultaneously record (with low artifacts) neuronal responses at up to 512 electrodes. The system is compatible with most silicon probes used in the brain research and can use up to four probes in parallel, providing bidirectional communication with populations of neurons simultaneously in several brain areas.

Each channel of the chip includes a recording amplifier and a stimulation circuit. The amplifier has adjustable gain (110-550x), low cut-off frequency (1.4-7 Hz), and anti-aliasing filter frequency (1.2-14 kHz). The input-referred noise is 6.8 μV. Signals from all the channels are digitized at 40 kHz. The stimulation signal is defined independently for each channel with 40 kHz refresh rate. The stimulation artifacts are reduced by temporally disconnecting the amplifiers from electrodes and optimization of the pulse waveform.

METHOD(S): The system has been tested in experiments exploring somatosensory thalamo-cortical network in rodents. 2-3 weeks before surgery, animals received injections of AAV-hSyn-ChR2-EYFP viral vector. In anesthetized animals, multichannel probes were inserted into the barrel cortex and/or sensory thalamus for recording of LFPs and multi-unit responses to

microstimulation delivered to various nodes of thalamo-cortical network. Electrically evoked activity was compared with responses to natural whisker deflection and optical stimulation.

RESULTS: The reported system can generate complex patterns of stimulation pulses and record neuronal signals with very low artifacts at up to 512 electrodes, making it a powerful tool for mapping of the functional connections between brain circuits.

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P5.4. INTRACELLULAR RECORDING OF MOUSE SPINAL MOTONEURONS IN VIVO

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INTRODUCTION: Spinal motoneurons represent the final common pathway of the motor system. They are not static elements of the network, but alterations in the levels of physical activity or pathological mechanisms are known to affect their electrophysiological properties. Sharp intracellular recordings are the firmly established gold standard for studying motoneuron excitation/inhibition patterns *in vivo* in adult animals; however, until recently this technique was not available for mice. We've set out to create an electrophysiological setup that would allow us to perform stable intracellular recordings of mouse motoneurons *in vivo* in order to take advantage of numerous genetic models available for this organism.

METHOD(S): Animals were anesthetized with a mix of fentanyl/medetomidine/midazolam, and a complex surgical procedure was performed, that included catheterization of the jugular vein, insertion of a tracheal tube, exposure of the triceps surae (TS) nerve, and Th13-L2 laminectomy. Sharp glass microelectrodes were inserted into the spinal cord using motorized micromanipulator, and TS nerve was stimulated with constant current pulses in order to evoke motoneuron antidromic activation. Upon successful penetration, TS motoneurons were identified based on an "all or nothing" appearance of action potential, and passive, threshold and synaptic properties were recorded using intracellular stimulation/amplifier. At the end of the experiment, mice were euthanized with overdose of barbiturates.

RESULTS: Our approach enabled us to record 2-5 motoneurons in a single experiment with stability ensuring precise measurement of passive membrane properties, intrinsic excitability, and synaptic excitation.

CONCLUSIONS: We prove the feasibility of performing stable intracellular recordings of mouse spinal motoneurons *in vivo*, which should pave the way for future studies of motoneuron plasticity under physiological or pathological conditions.

FINANCIAL SUPPORT: This work was supported by NCN grant no. 2017/26/D/NZ7/00728.

P5.5. PYECOHAB: A PYTHON LIBRARY FOR ANALYSIS OF RODENT BEHAVIORAL DATA RECORDED WITH ECO-HAB

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INTRODUCTION: How can we make mouse studies more reproducible? The obvious answer is the standardization of experimental conditions, minimization of human interference, automation of behavioral tests and data analysis, and introduction of data analysis pipelines to automate the process. Eco-HAB, a system for automated measurement of social preference and in-cohort sociability in mice, provides a solution for the first two issues. Eco-HAB closely follows murine ethology, providing a 4-compartment apparatus with narrow tunnels, and minimizes contact between the experimenter and tested animals.

Introduction of pyEcoHAB, a Python library for analysis of EcoHAB murine behavioral data.

AIM(S): pyEcoHAB, a Python package, has been developed to automate and facilitate data analysis.

METHOD(S): Combining data access and initial interpretation, pyEcoHAB removes the need to do this manually, and allows the researcher to build data analysis pipelines and automation of behavioral tests facilitating data interpretation. pyEcoHAB provides an object-oriented application programming interface (API) and a data abstraction layer. Auxiliary utilities supporting development of analysis workflows are integrated with pyEcoHAB, including data validation and workflow configuration tools. Moreover, pyEcoHAB provides methods for assessment of mice social behavior, such as approach to social odor, total time spent by each pair of mice together in each compartment (in-cohort sociability), number of times each mouse follows other mice in narrow tunnels (following), and also, the number of times each mouse pushes other mice out of a narrow tunnel. The latter behavior is similar to tube dominance tests and is an example of how traditional behavioral tests can be automated.

CONCLUSIONS: pyEcoHAB is a computational framework facilitating automatic analysis of behavioral data from EcoHAB system.

FINANCIAL SUPPORT: This work was supported by the Polish National Science Centre grant 2017/27/B/NZ4/02025.

P5.6. KERNEL ELECTRICAL SOURCE IMAGING (KESI) METHOD FOR RECONSTRUCTION OF SOURCES OF BRAIN ELECTRIC ACTIVITY IN REALISTIC BRAIN GEOMETRIES

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INTRODUCTION: Around 50 million people worldwide are affected by epilepsy. Despite efficiency and steady development of pharmacological treatments, every third patient suffers from intractable seizures. A surgical intervention may be the only solution in these cases. To identify the region for resection, neurosurgeons implant intracranial and subdural electrodes which are used to localize the epileptogenic zone from the measured potentials.

AIM(S): Providing better tools for reliable reconstruction of sources of brain activity may lead to more precise localization of the seizure's origin and better surgical outcomes. To reconstruct sources of brain activity, we use kernel approximation methods for the inverse problem (the reconstruction itself). We model the electric field generated by the neural activity (the forward problem) with finite element method (FEM). We use FEM as it enables the inclusion of realistic head anatomy and tissue properties in the model.

METHOD(S): Here we present a method – kernel Electrical Source Imaging (kESI) – of reconstruction of the activity underlying the measured potentials. kESI allows us to use information from arbitrarily placed electrodes and may integrate patient-specific anatomical information which increases precision of localization of epileptogenic zone for a specific patient.

RESULTS: The preliminary results are promising. The major advantage of kESI over previous work is that it accounts for spatial variations of brain conductivity and can take into account patient-specific brain and skull anatomy.

CONCLUSIONS: Nevertheless, further work is necessary to bring this method to the level of clinical application.

FINANCIAL SUPPORT: Project funded from the Polish National Science Centre's OPUS grant (2015/17/B/ST7/04123).

P5.7. ASSESSMENT OF BIOMARKERS OF ATTENTION IN MODIFIED DELAY MATCH-TO-SAMPLE TEST

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INTRODUCTION: Information processing and stimuli filtering mechanisms are dispersed over many networks engaged in different processes and located all over the brain. In the present study, we examined neural correlates of attention-related working memory during the repeated modified delay match-to-sample test (DMTS).

AIM(S): To find the most effective analytical method for assessment of attention related activity to be used in neurofeedback training.

METHOD(S): In order to identify neuronal activity underlying state of increased attention, we used a DMTS test amended by control trials that did not require the engagement of attention and memory. These additional trials allowed us to compare the impact of attended versus passive conditions on electrical brain activity. We examined 14 subjects in 3 sessions performed within 10-20 days. EEG was collected with 21 electrodes in the 10-20 system. For each electrode channel and trial in selected time windows, we analyzed power in consecutive frequency bands (<40 Hz) and signal complexity measures, including sample entropy, Shannon entropy, and Higuchi fractal dimension (HFD).

RESULTS: The results averaged over the whole group showed significant differences between EEG signals recorded during attentional and control trials on several electrodes. However, at the level of individual subjects, the selection of signals with such differences varied between subjects and applied methods. The most prominent effect of attention was observed in a window extracted from the 5-sec period of stimulus expectation and information retention, not accompanied by sample of the object. With repetition of DMTS sessions, the effect of differentiation of attentional and controls trials has been also emphasized in all analyzed measures.

CONCLUSIONS: The results indicated the importance of the individual subject and session analysis and relevance of applying signal complexity methods to support spectral analysis in a further application.

P5.8. KERNEL CURRENT SOURCE DENSITY REVISITED

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INTRODUCTION: Extracellular recordings reflect transmembrane currents of neural and glial cells and thus have long been the foundation of measurements of neural activity. Recorded potential reflects activity of the underlying neural network and is directly related to the distribution of current sources along the active cells (current source density, CSD). The long-range of the electric field leads to significant correlations between recordings at distant sites, which complicates the analysis. However, data interpretation can be facilitated by reconstruction of current sources.

AIM(S): Facilitate reconstruction of sources of brain activity with open software.

METHOD(S): The Kernel Current Source Density method (KCSD) is a general non-parametric framework for CSD estimation based on kernel techniques, which are widely used in machine-learning. KCSD allows for current source estimation from potentials recorded by arbitrarily distributed electrodes. Overfitting is prevented by constraining complexity of the inferred CSD model.

RESULTS: Here, we revisit KCSD to present a new, open-source implementation in the form of a package, which includes new functionality and several additional tools for kCSD analysis and for validation of the results of analysis accompanied by extensive tutorials implemented in Jupyter notebook. Specifically, we have added 1) analysis of spectral properties of the method; 2) error map generation for assessment of reconstruction accuracy; and 3) L-curve, a method for estimation of optimum reconstruction parameters. The new implementation allows for CSD reconstruction from potentials measured by 1D, 2D, and 3D experimental setups for a) sources distributed in the entire tissue, b) in a slice, or c) in a single cell with known morphology, provided that the potential is generated by that cell.

CONCLUSIONS: New Python implementation of kCSD facilitates CSD analysis and allows for estimation of errors. The toolbox and tutorials are available at https://github.com/Neuroinflab/kCSD-python.

P6.1. A LINK BETWEEN GLUTAMINE METABOLISM AND SELECTIVE NEURONAL VULNERABILITY IN THE HIPPOCAMPUS

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INTRODUCTION: The brain has developed several endogenous mechanisms to protect itself from the harmful consequences of ischemia/reperfusion (I/R) injury. Understanding of such mechanisms may be important for the development of new neuroprotective therapies. In a gerbil model of cerebral ischemia, 5 min-ischemia results in selective, delayed neuronal death in the hippocampal CA1 region, while CA2-4 and the DG remains relatively resistant. We have shown that I/R-induced translocation of protein kinase C beta II (PKCβII) from cytoplasm to mitochondria only in CA2-4 and the DG is relevant for ischemia-resistance of these regions. The exact mechanism remains unknown.

AIM(S): The aim of the study was to investigate the role of kidney-type glutaminase (GLS1), identified as a potential PKCβII partner, in PKCβII-mediated neuroprotection.

METHOD(S): Reciprocal co-immunoprecipitation method showed that of the two GLS1 isoforms, it is GAC not KGA that interacts with PKCβII. *In vitro* studies revealed that GLS1 may be phosphorylated by PKCβII. GLS1 converts glutamine to glutamate, thus the effect of I/R on activity of GLS1 and level of glutamine and glutamate in mitochondria-enriched fraction were measured.

RESULTS: Glutaminase activity is higher in CA2-4 and DG as compared to CA1 in control and 1 h after I/R, and is confirmed by a reduced level of glutamine in this region. Glutamate level seems to be similar in both regions and is not affected by I/R injury. Application of a selective PKCβII inhibitor increased GLS1 activity in both regions.

CONCLUSIONS: This indicates that GAC is not relevant in PKCβII-mediated neuroprotection, however PKCβII seems to have an influence on the maintenance of glutaminase activity. Moreover, we speculate that ischemia-resistance of CA2-4 and the DG is due to its high glutaminase activity, which provides a large amount of glutamate that, in turn, can be effectively used for ATP production in the Krebs cycle or for antioxidant defense based on glutathione synthesis.

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P6.2. LOOP G OF THE GABAA RECEPTOR ORTHOSTERIC BINDING SITE IS INVOLVED IN THE FINAL STAGES OF CHANNEL GATING

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INTRODUCTION: Inhibition of neuronal activity is shaped primarily by $GABA_A$ receptors. Agonist binding

site (BS) at the β+/α- intersubunit interface is composed of 7 loops (A-C from β and D-G from α subunit), and the Loop G has been reported to play a major role in receptor activation, however the exact mechanism is not clear. α_1F45 residue at Loop G has been shown to be engaged in receptor activation despite not directly contacting the agonist, and is well positioned for interactions with other crucial BS residues. Since this loop spans from the BS to the extracellular-transmembrane domain interface, it might have an important role in transferring energy of BS conformational transitions to the pore region.

AIM(S): This study aims to reveal the role of loop G in distinct steps of receptor activation.

METHOD(S): We used rapid agonist application to elicit macroscopic responses and single-channel recordings of GABA-evoked currents for wild-type (WT) and mutated (α_1F45C/L/K/G) receptors. Model simulations of macroscopic and single-channel activity and *in silico* structural analysis have been performed.

RESULTS: Mutated receptors showed a different kinetic profile of macroscopic currents (except α_1F45L) with faster deactivation (α_1F45C/K/G) and impaired desensitization (α_1F45C/G). Single-channel currents showed profound differences in all mutants; that is, closures were prolonged, openings were shortened, and P_{open} within bursts was reduced. Model simulations revealed changes primarily in opening/closing transitions. The homology model of WT showed loop G energy minimum at the α_1F45 position, underlining its role in loop stability. In α_1F45G/K mutants, this minimum declined. In α_1F45G mutant, it can be attributed to the BS aromatic box disruption and α_1F45K substitution could impair the GABA – α_1R66 interaction.

CONCLUSIONS: Mutations of the α_1F45 residue in loop G of the BS affects final gating stages. This indicates the role of loop G in linking binding and gating processes.

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P6.3. MATRIX METALLOPROTEINASE 3 CRITICALLY AFFECTS POSTSYNAPTIC LONG-TERM POTENTIATION AT GABAERGIC SYNAPSES IN THE MOUSE HIPPOCAMPAL CA1 REGION

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INTRODUCTION: Matrix metalloproteinases (MMPs) are extracellular proteases that play a crucial role in various forms of neuronal plasticity. We have recently shown that MMP-3 supports NMDAR-mediated long-term potentiation and L-type calcium channel-dependent LTP. An extensive body of evidence revealed that, besides glutamatergic transmission, GABAergic synapses are also plastic; however, the underlying mechanisms remain elusive.

AIM(S): Herein we addressed the question if activity of MMP-3 is involved in GABAergic synaptic plasticity in mice acute hippocampal slices.

METHOD(S): We performed whole-cell patch-clamp recordings of miniature inhibitory postsynaptic currents (mIPSCs) from hippocampal CA1 pyramidal neurons. To induce iLTP, we applied NMDA in bath solution (3 min, 20μM) in the presence of 20 μM DNQX and 1μM TTX to slices from wild-type (WT) animals and mice lacking the *mmp-3 gene* (MMP-3 KO). To block the activity of MMP-3, we used inhibitor UK 356618 (2 μM) in different time windows upon iLTP induction.

RESULTS: We found that, in contrast to control conditions (WT), iLTP evoked in MMP-3 KO mice was completely abolished (CTR: 122±8%, n=9; MMP-3 KO: 99±4%, n=13; $p<0.05$). We next studied the impact of the MMP-3 inhibitor (UK356618) on iLTP during different time windows. The application of the MMP-3 inhibitor before induction blocked iLTP (UK 356618: 92±3%; n=7; CTR: 116±3%; $p<0.05$). In slices that were treated with UK 356618 at various time points after starting the NMDA application, we found that the activity of MMP-3 is required for up to 13 minutes post induction of iLTP (UK 356618: 113±2%; n=6; CTR: 116±3%; n=7; $p>0.05$).

CONCLUSIONS: The present results provide evidence that the activity of MMP-3 plays a crucial role in iLTP in the hippocampal CA1 region within a specific time window.

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P6.4. LAMOTRIGINE AFFECTS THETA OSCILLATIONS IN THE HIPPOCAMPUS OF RATS

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INTRODUCTION: Neuronal synchronization depends on many factors including HCN channel action. They are voltage-gated ion channels that mediate an inward cationic current dependent on hyperpolarization. There is sparse evidence for their contribution to neuronal plasticity, learning and memory, epilepsy, or

Alzheimer's disease. HCN channels can be found in the hippocampus (HPC) and are thought to be involved in neuronal synchronization through initiating membrane potential oscillations which are necessary for the appearance of field theta oscillations. Hippocampal theta rhythm is known to be involved in memory formation, spatial navigation, sensorimotor integration, movement initiation, and others. So far it is established that HPC theta generation is a result of a fine balance between the cholinergic and GABAergic system activation, which triggers the synchronous action of theta-related neurons. However, the involvement of HCN channels in this process is still mostly unknown.

AIM(S): The aim of this study was to investigate the role of HCN channel activation in the process of theta generation.

METHOD(S): Three experimental models were used: *in vivo* anesthetized rats, *in vitro* acute HPC slices, and HPC patch clamp whole cell method. Field and single neuron recordings were made from the HPC after perfusion with a non-specific HCN channels agonist – lamotrigine (LTG).

RESULTS: When LTG was applied it produced mixed results. In particular, it blocked theta rhythm *in vitro* but significantly enhanced it *in vivo*. Patch clamp results have shown that LTG reduced the frequency of spontaneous inhibitory postsynaptic currents but also decreased the excitability and membrane resistance of CA1 neurons. Also, LTG reduced membrane potential theta resonance in most CA1 cells.

CONCLUSIONS: HCN channels activation was shown to have an impact on the process of theta rhythm generation in the HPC. Current results are discussed.

FINANCIAL SUPPORT: Supported by National Science Centre, grant no. 2017/26/D/NZ4/00159.

P6.5. CHARACTERISTICS OF RAT VENTRAL TEGMENTAL AREA GABAERGIC-LIKE NEURONAL RESPONSES TO AN AVERSIVE STIMULUS

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INTRODUCTION: The ventral tegmental area (VTA) is a major source of dopamine in the mammalian brain. Previous studies have confirmed that the pattern of VTA dopamine (DA) cell spontaneous activity is tonic rather than phasic during an activation state in contrast to deactivation (SWA), generated by urethane anesthesia. However, little is known about the second largest neuronal population in the VTA – GABA-ergic cells, which best known functional role is to inhibit the surrounding DA neurons, for e.g., in response to an aversive stimulus.

AIM(S): The aim of our study was to investigate how VTA non-DA neurons respond to an aversive stimulus (footshock) and whether this reaction changes with an alterations in the brain states elicited by urethane anesthesia application.

METHOD(S): We performed extracellular *in vivo* recordings of non-DA neurons combined with simultaneous recording of local field potentials from the hippocampus. Recordings were performed using SD-TH-Cre$^{+/-}$ rats under urethane anesthesia and electric footshocks were applied (45 per state) to the hindpaw of the animal in both brain states. We used optogenetics to identify the phenotype (DA *vs.* non-DA cell phenotype) of the footshock responsive cells by photo-tagging.

RESULTS: GABAergic-like (non-DA) cells respond to the footshock with either excitation (36%) or inhibition (16%) of their activity regardless of the brain states. Interestingly, a fraction of those non-DA cells (20%) reacted with inhibition during activation and excitation during a deactivation state. The rest of the cells react with no-responses in at least one state (28%).

CONCLUSIONS: In line with previous studies, we observed a population of non-DA cells that react with excitation in response to footshocks. Interestingly, we also observed non-DA cells that were inhibited and populations that reacts depending on current brain state. Our results indicate a more complex role of GABAergic cells in response to footshock than previously assumed.

P6.6. THE RESPONSE OF MAIN SUBCLASSES OF GABAERGIC INTERNEURONS TO PLASTICITY INDUCTION AND AGING: CHANGES IN MRNA LEVELS

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INTRODUCTION: Inhibitory interneurons undergo age-related alterations that may have tremendous consequences on cellular and network computations and account for cognitive and behavioral deficits. Accordingly, we have shown that mechanisms governing fear learning-induced plasticity were weakened in aged (1 y.o.) mice somatosensory cortex, hampering manifestation of plastic changes, while in old (2 y.o.) mice the plasticity was absent.

AIM(S): To investigate age-related mRNA changes of distinct markers that are characteristic of GABAergic interneurons, define their main subtypes, and correlate potential changes with age-related plasticity impairments.

METHOD(S): Plasticity was induced with a classical conditioning paradigm, in which tactile stimulus to one row of whiskers was paired with a tail electric shock. Three groups of mice were used: young (3 months old), aged (1 y.o.) and old (2 y.o.). Using qRT-PCR, we investigated mRNA levels of GAD67, GAD65, parvalbumin (PV), somatostatin (SST), calretinin (CR), calbindin (CB), vasoactive intestinal polypeptide (VIP), and Neuropeptide Y (NPY).

RESULTS: qRT-PCR analysis showed changes in mRNA levels, resulting from both aging itself and from plasticity induction. mRNA level of CB decreased in aged and old animals, whereas PV increased in the old group. After plasticity induction, we observed a reduction of NPY in the young group, while aged animals presented a decline of VIP mRNA levels.

We observed decrease in CB along with an increase in PV mRNA levels, which may result in calcium homeostasis disruption in neurons and may consequently be involved in the plasticity impairments observed in aged and old animals.

CONCLUSIONS: Being a part of the VIP-SST disinhibitory circuit that exist in many cortical areas, VIP mRNA changes may contribute to dysregulation of this important mechanism controlling plasticity.

FINANCIAL SUPPORT: National Science Centre grant 2013/09/B/NZ3/00540.

P6.7. DECODING THE SPONTANEOUS *IN VIVO* Ca^{2+} OSCILLATIONS IN ZEBRAFISH BRAIN NEURONS

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INTRODUCTION: Neurons code sensory information in Ca^{2+} spiking trains at discrete points in time and facilitate synaptic plasticity and synchronicity in the neurons. Ca^{2+} can enter the cytoplasm through gated ion channels. One of the regulatory mechanisms of Ca^{2+} homeostasis in neurons is Store-operated Ca^{2+} entry (nSOCE). When Ca^{2+} is depleted from the endoplasmic reticulum (ER: a major store of Ca^{2+}), the decreased level of Ca^{2+} is sensed by STIM proteins (ER residents), which then oligomerize and interact with Ca^{2+} channels at the plasma membrane. This leads to Ca^{2+} influx and refilling ER with these ions by Ca^{2+}-ATPase.

Analysis of parameters of the neuronal spontaneous Ca^{2+} oscillations *in vivo* and those in response to the external stimuli. Zebrafish larva is an exceptional model for whole-brain functional imaging. However, the mechanisms underlying the spontaneous neuronal Ca^{2+} activity patterns, and their biological relevance, remain elusive.

METHOD(S): Using Lightsheet Microscopy, we performed *in vivo* imaging of transgenic fish expressing GCaMP5G (a genetically encoded Ca^{2+} probe) under the neuronal promoter (*Tg(elavl3: GCaMP5*). The parameters of Ca^{2+} oscillations, such as the interspike intervals (ISI) and the Ca^{2+} amplitudes, were analyzed in neuronal somata of the three different regions of the brain (optic tectum (OT), cerebellum (Cereb), and inferior olive (IO)) of transgenic (TG), wild-type (WT), and *stim2b*$^{-/-}$ zebrafish lines.

RESULTS: Using MATLAB algorithms, we quantified the differences in Ca^{2+} oscillations patterns between regions in the brain neurons and showed that Ca^{2+} oscillations change significantly in s*tim2b*$^{-/-}$ fish. ISI was reduced, and Ca^{2+} amplitude was increased in s*tim2b*$^{-/-}$ as compared to transgenic WT in fish treated with a high concentration of Ca^{2+}.

CONCLUSIONS: This Ca^{2+} spiking trains modulation by Stim2b protein suggests its role in attenuation of Ca^{2+} buffering.

P6.8. EXPRESSION OF CANNABINOID RECEPTOR TYPE-1 IN DIFFERENT SUBPOPULATIONS OF KISSPEPTIN NEURONS IN THE MOUSE BRAIN

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INTRODUCTION: Kisspeptin (KP) is involved in multiple hypothalamic regulatory processes such as estrogenic feedback to GnRH neurons via synaptic connections. We have reported previously that GnRH neurons send endocannabinoid retrograde signals to regulate their GABAergic input. KP-producing neurons co-synthesize GABA or glutamate both in the preoptic (POA) and arcuate (ARC) subpopulations. Moreover, we found the cannabinoid receptor type one (CB1) mRNA in these areas. However, it is unknown whether any subpopulation of KP neurons is under the influence of endocannabinoids.

AIM(S): 1) To identify the GABAergic and glutamatergic subpopulations of KP neurons in female mice. 2) To determinate whether CB1 transcripts can be detected in any of the subpopulation of POA or ARC KP neurons.

METHOD(S): The transcripts of KP, CB1, and the GABAergic and glutamatergic marker proteins, i.e., vesicular inhibitory amino acid transporter (VIAAT), and vesicular glutamate transporter-2 (VGLUT2) have been detected by RNAscope *in situ* hybridization technique. The signal was analyzed by confocal microscopy and quantified using our in-house developed program.

RESULTS: We found a significantly increased KP mRNA expression in the POA of ovariectomised mice after estradiol replacement. CB1 mRNAs were found in GABAergic and glutamatergic KP neurons of the POA and ARC. About 67% (AVPV) – 43% (ARC) of the GABAergic KP neurons expressed CB1 mRNA in the OVX+OIL mice, and about 31% (AVPV) – 36% (ARC) of the GABAergic KP neurons expressed CB1 mRNA in the OVX+EB mice. Concerning the glutamatergic KP neurons, about 19% (AVPV) -14% (ARC) expressed CB1 mRNA in the OVX-+OIL mice and about 24% (AVPV) – 43% (ARC) of them expressed CB1 mRNA in the OVX+EB mice.

CONCLUSIONS: The hormonal status of mice influences the number of KP neurons expressing CB1 and endocannabinoids likely regulate the electrical activity of different KP subpopulations involved in the regulation of various hypothalamic processes.

P6.9. INCREASE OF INTRINSIC EXCITABILITY OF NEOCORTICAL SOMATOSTATIN-EXPRESSING CELLS IN LAYER IV OF THE MOUSE PRIMARY SOMATOSENSORY CORTEX AS A RESULT OF ASSOCIATIVE LEARNING

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INTRODUCTION: GABAergic (inhibitory) interneurons are critical for information processing in the brain during learning and memory and also undergo learning-dependent plastic changes. However, the exact mechanisms of learning-evoked changes in the GABAergic system are not fully explored. Inhibitory interneurons constitute about twenty percent of all cortical neurons and are highly heterogeneous, creating functional classes based on their molecular, electrophysiological, and morphological features, as well as connectivity and patterns of activity. According to molecular markers, three main groups of interneurons were discovered in the neocortex: SST (somatostatin-), PV (parvalbumin-), and VIP (vasoactive intestinal polypeptide-) expressing cells.

AIM(S): The aim of the project was to study effects of associative learning on SST interneuron activity in the somatosensory cortex of mice.

METHOD(S): Transgenic mice with fluorescently labeled SST interneurons were subjected to a conditioning procedure in which whisker stimulation was paired with a tail shock. As a control group, we used naïve mice and mice subjected to stimulation of vibrissae and a tail shock given at random relative to whisker stocking (pseudoconditioning). After learning, we prepared acute brain slices and performed whole-cell patch-clamp recordings in SST interneurons of layer IV in the cortical representation of the whiskers stimulated during the learning protocol.

RESULTS: We found an increase in intrinsic excitability of SST interneurons after conditioning. Spontaneous activity of SST neurons as well as sEPSCs recorded in SST neurons were similar between groups.

CONCLUSIONS: Our results suggest that the increase in SST intrinsic excitability is a common mechanism of plastic changes after learning. Literature data shows that learning increases intrinsic excitability of hippocampal SST interneurons.

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P6.10. NEUROGENESIS AND BEHAVIORAL STRATEGIES IN ICER OVEREXPRESSING RATS

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INTRODUCTION: ICER (Inducible cAMP Early Repressor) is an effective endogenous repressor of CREB/CREM/ATF transcription factors family, including its own expression. We have developed a Syn-Flag-ICER II transgenic rat line. In transgenic animals, we have surprisingly detected increased levels of mRNA for CREB or CREM transcription factors. We have also detected upregulation of CREB dependent miR-132.

Nerogenesis is a process of generation and maturation of newborn neurons into neuronal networks in the developing brain. We have found that ICER II overexpressing rats showed reduced hippocampal adult neurogenesis. The number of the SGZ BrdU positive cells was similar, but in the mature granular neurons layer, the number of BrdU positive cells was decreased when compared to control animals. One of the crucial elements enabling the incorporation of newborn neurons into neuronal network of the brain is the active reorganization of the extracellular matrix mostly by action of metalloproteinases. The most known for its activity in the brain is matrix metalloproteinase 9 (MMP-9), which is also one of the known targets of miR-132. We have examined MMP-9 activity in the ICER overexpressing rat brain lysates, and we observed decreased activity of MMP-9 in ICER mutants as compared to WT controls.

RESULTS: We have also found that ICER rats with affected neurogenesis employ different learning strategies than their control littermates in the Morris Water Maze learning paradigm. The results of this behavioral tests indicate that transgenic rats didn't differ from controls in

their learning and memory capabilities, but they showed differences in strategies of finding the hidden platform. Male ICER rats more often were choosing imprecise strategies to find the platform than control males.

CONCLUSIONS: Those results demonstrate that disruption of CREB dependent gene expression in neurons by overexpression of ICER affects adult neurogenesis and causes changes that affect discrete aspects of animal cognitive behavior.

P6.11. POSTERIOR HYPOTHALAMIC "TIMING CELL" ACTIVITY AND KAINITE-INDUCED LOCAL THETA RHYTHM IN BOTH *IN VITRO* AND *IN VIVO* PREPARATIONS

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INTRODUCTION: Theta rhythm typically occurs during memory processes, REM sleep, and spatial navigation but also in epilepsy, migraines, or even mild Alzheimer's disease (AD). Recent evidence shows that well-synchronized theta rhythm can successfully be recorded locally from the posterior hypothalamic area (PHa), specifically from the supramammillary nucleus (SuM) and the posterior hypothalamic nuclei (PH). The population of theta-related cells in the PHa were found to be similar types to those found in the hippocampal formation. In addition, a new type of cells has been found in the posterior hypothalamic region and based on its regular firing pattern and possible pacemaker role these cells were termed "timing".

AIM(S): The aim of the present study was to investigate the timing of cell populations in both *in vivo* and *in vitro* PHa after theta rhythm induction by kainic acid (KA) application.

METHOD(S): Twenty *in vivo* experiments were performed on 20 urethanized rats and 22 *in vitro* experiments were performed on 40 PHa slices obtained from 22 rats. Theta rhythm and single unit activity were evoked by intra-PHa microinjection of KA (*in vivo*) or by bath perfusion of PHa slices with KA-containing artificial cerebrospinal fluid (*in vitro*).

RESULTS: A total number of 123 posterior hypothalamic neurons were recorded during both *in vivo* and *in vitro* experiments. Among them, 28 neurons were classified as "timing cells" according to their very regular pattern of discharges in a steady frequency in the theta band (3-12 Hz). Eight timing cells were recorded in *in vivo* PHa and 20 timing cells were recorded in PHa slices.

CONCLUSIONS: The present data show that glutamatergic stimulation of PHa neuronal network with kainic acid results in the activation of specific subpopulation of neutrons, characterized by regular firing pattern in theta frequency range. The role of PHa "timing cell" activity is discussed regarding hippocampal theta rhythm frequency programing.

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P6.12. GABAERGIC PLASTICITY IN HIPPOCAMPUS DEPENDS ON THE ACTIVITY OF MATRIX METALLOPROTEASE-3

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INTRODUCTION: Formation of memory traces, and thus learning, strongly relies on the interplay between different forms of neuronal plasticity. While much is known about the plasticity of glutamatergic synapses, plasticity of synaptic inhibition awaits deeper investigations.

AIM(S): The aim of this study was to address the function of extracellular proteolysis mediated by matrix metalloprotease-3 (MMP-3) in GABAergic plasticity.

METHOD(S): We recorded mIPSCs in hippocampal culture and induced inhibitory LTP (iLTP) using NMDA in the presence of MMP-3 inhibitor UK-356618. Additionally, we studied changes in morphology of GABAergic synapses and membrane lateral mobility of $GABA_A$Rs.

RESULTS: Obtained results clearly show that, in the presence of UK or in MMP-3 deficient neurons, iLTP is abolished (WT: 116%; UK: 96%, n=8-9, p=0.02). Concurrently, we observed a significant increase in synaptic gephyrin cluster area after iLTP induction in controls (WT: 121%, n=23) but not in the presence of UK (98%, n=25, p<0.01). We also investigated the effects of exogenous active MMP-3 on synaptic morphology, membrane mobility of $GABA_A$ receptors, and the amplitude of mIPSC. Short-term MMP-3 application augments mIPSC amplitude up to 121% of initial value (n=11, p<0.01) and increases the average size of synaptic gephyrin cluster (108%, n=16, p<0.05). In addition, analysis of membrane mobility of synaptic $GABA_A$Rs showed a decrease in their diffusion coefficient after MMP-3 treatment (before: 0.019μm^2/s; after MMP-3: 0.015μm^2/s, p<0.01) indicating the strengthening of inhibitory synapses through receptor trapping.

CONCLUSIONS: These results demonstrate a crucial role of MMP-3 in the induction of iLTP both at functional and morphological level opening new avenues in the study of plasticity cross-talk between different synapses. Additionally, presented results significantly expand

our knowledge on the local interplay between extracellular matrix and inhibitory synapses.

FINANCIAL SUPPORT: NCN grant 2017/26/D/NZ4/00450.

P6.13. THE TRANSCRIPTION FACTOR TCF7L2 GOVERNS POSTMITOTIC EMBRYONIC DEVELOPMENT OF THE THALAMUS AND ADULT INTRINSIC EXCITABILITY OF THALAMIC NEURONS

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INTRODUCTION: The thalamus integrates sensory information and is involved in the selection of behavioral responses. This requires proper development of thalamic nuclei, thalamocortical connections, and electrophysiological properties of thalamic neurons. Molecular mechanisms of postmitotic thalamic differentiation and adult homeostasis were poorly characterized. Our studies show that both are regulated by the transcription factor TCF7L2.

AIM(S): To determine the role of TCF7L2 in the development of thalamic cytoarchitecture, molecular anatomy, thalamocortical connections, and intrinsic excitability of thalamic neurons.

METHOD(S): We examined mouse embryos (E18.5) with a total knockout of *Tcf7l2*, and adolescent/adult mice (P20-P60) with thalamus-specific, postnatal knockout of *Tcf7l2*. Embryonic brain slices were used for Nissl staining to visualize anatomical structures, *in situ* hybridization for gene expression analysis, immunohistochemistry to visualize axon fibers and diencephalic substructures, or thalamocortical neural tracts tracing with DiI. Comparative RNA-seq analysis was performed on isolates from thalami of both mouse strains. Live brain slices from adolescent TCF7L2-deficent mice were used for *in vitro* patch-clamp analysis of thalamic neurons.

RESULTS: E18.5 *Tcf7l2*$^{-/-}$ mice show changes in anatomical and molecular boundaries in diencephalon, fail to produce thalamocortical axons, and do not maintain the expression of main transcription factors that mark thalamic subregions. Postnatal TCF7L2-deficent thalamic neurons show reduced burst and tonic spiking.

CONCLUSIONS: Accordingly, RNA-seq study revealed changes in the expression of their typical ion channels. TCF7L2 orchestrates a network of transcription factor genes to regulate postmitotic molecular differentiation, segregation of neurons, and axon path-finding in the thalamo-habenular domain. Continuous expression of TCF7L2 in adult is required to establish proper intrinsic electrophysiological properties of thalamic neurons.

POSTER SESSION 2

P7.1. TIME INCREASES THE ODDS OF REPEATING A PREVIOUS CHOICE

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INTRODUCTION: Reinforcement learning causes an action that produced a satisfying effect in a particular situation to become more likely to occur again in that situation. The process is essential in adaptive behavior; however, actual choices often appear to diverge from what could be inferred from simple reinforcement learning.

AIM(S): Here we investigate how the time intervals between actions affect the choices made.

METHOD(S): Groups of C57BL6/J mice were housed in IntelliCages with access to water and chow *ad libitum* and were able to access bottles with a reward in the form of a saccharin solution (0.1% w/v), alcohol (4% w/v), or a mixture of the two. The probability of receiving a reward in two of the cage corners changed to 0.9 or 0.3 every 48 h over a period of ~33 days.

RESULTS: We observed that, in most animals, the odds of repeating the choice of a corner were increased if that choice was previously rewarded. Interestingly, the time elapsed from the previous choice also increased the probability of repeating the choice, irrespective of the previous outcome. Behavioral data were fitted with a series of reinforcement learning models based on Q-learning. We found that introducing an interval-dependent adjustment allowed for better description of the observed behavior, and the size of the time effect differed depending on the type of reward offered.

CONCLUSIONS: We find that, at longer time intervals, repeating the previous choice becomes more probable, irrespective of the previous outcome. Thus, at least in this specific case, time may make a past mistake more likely to be repeated.

P7.2. INHIBITION AND ACTIVATION OF THE CENTRAL AMYGDALA CIRCUITS INVOLVED IN SOCIAL INTERACTION SUPPRESSES MOTIVATION FOR SUCROSE REWARD

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INTRODUCTION: Appetitive motivation systems evolved to mediate a wide array of adaptive behaviors aimed at providing resources such as food or social contacts.

AIM(S): The question whether motivation to approach various types of reward involves different neuronal mechanisms is still largely unanswered. In particular, it is not known whether neuronal circuits controlling social motivation are uniquely social, i.e., do they apply only to the social domain and are not utilized by other non-social motivational processes?

METHOD(S): To address this question, we manipulated activity of the central amygdala (CeA) circuits activated during either instrumental conditioning for food reward or interaction with a partner.

CeA has been implicated in generating intense incentive motivation for food and drugs. Using *c-fos*-driven targeting with halorhodopsin and channelorhodopsin, we were able to inhibit or activate the respective neuronal subpopulations in the CeA during the Skinner box session, in which motivation was assessed in the progressive-ratio schedule of food-pellet reinforcement. To obtain food pellets rats, had to press the lever. The number of responses required to get reinforced increased when the reward was obtained. Motivation was measured as the highest number of responses performed to obtain the food reward.

RESULTS: We observed that both inhibition and activation of either the social or food neuronal circuits in the CeA resulted in significantly decreased motivation for sucrose reward; however, the pattern of behavioral responses observed after manipulation of sucrose- and social-related neuronal circuits was different.

CONCLUSIONS: The results suggest that social and food motivation depends on circuits that overlap only partially.

P7.3. INVESTIGATION INTO THE EFFECTS OF CHRONIC ANTIDEPRESSANT AND ACUTE KETAMINE TREATMENT ON ULTRASONIC VOCALISATIONS IN WISTAR-KYOTO AND SPRAGUE-DAWLEY RATS

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INTRODUCTION: The emission of ultrasonic vocalisation (USVs) is thought to be a major means of rat communication that can be affected by a wide range of stimuli. 55 kHz USVs are emitted in response to various positive/pleasurable situations and are considered to reflect an underlying positive affective state. Wistar-Kyoto (WKY) rats are an endogenous model of treatment-resistant depression (TRD), resistant to selective serotonin reuptake inhibitors (SSRIs) but responsive to ketamine.

AIM(S): The aims of this study were firstly to compare baseline USVs emission in WKY rats to an outbred Sprague-Dawley (SD) strain and to evaluate the effect of different classes of antidepressant drugs on USVs profile.

METHOD(S): WKY and SD rats were subjected to chronic vehicle (NaCl 0.9%, s.c.), fluoxetine (10 mg/kg, s.c.), desipramine (10 mg/kg, s.c.), or acute ketamine (5 mg/kg, s.c.) treatment followed by USVs recording in the home cage. An additional group of non-injected rats was included in the study. All recordings (Sonotrack, Metris) were taken for a total of 5 min at four different time points: 30 min (no ketamine), 24 h, 7 days, and 21 days of administration. A number of 55 kHz calls was analysed.

RESULTS: Overall, WKY rats emitted more 55 kHz calls when compared with SD rats. Chronic treatment with a commonly used antidepressant, fluoxetine, resulted in a persistent decrease in the number of "positive" calls at all time points when evaluated in WKY rats. In SD rats, a similar decrease was observed only during the first two days of drug administration. Chronic desipramine treatment had no effect on the number of 55 kHz calls, in either strain, when compared to vehicle group. Acute ketamine administration to WKY rats resulted in a decrease in a number of "positive" calls at 24 h but not at 7 or 21 days post-administration.

CONCLUSIONS: This study demonstrates a potential application of USVs recording as a relevant behavioural parameter that can be included in studies that investigate the effect of long-term and acute drug exposure on changes in emotionality.

P7.4. MICE WITH A DELETION OF THE MAMMAL--SPECIFIC MICRORNA 379-410 CLUSTER DISPLAY ALTERED ULTRASONIC COMMUNICATION

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INTRODUCTION: microRNAs (miRNAs) represent a group of small, noncoding RNA molecules that play

a major role in the posttranscriptional regulation of gene expression. Members of a large placental mammal-specific miRNA cluster, miR379-410 have been implicated in a variety of neurodevelopmental disorders. Recently, we have shown that deletion of this cluster in mice leads to hypersocial behavior and increased emission of ultrasonic vocalizations (USV), which is accompanied by altered excitatory synaptic transmission and exaggerated expression of ionotropic glutamate receptor complexes in the hippocampus.

To further investigate the contribution of the miR379-410 cluster to communication deficits present in neurodevelopmental disorders, here we performed a detailed analysis of acoustic features of isolation-induced pup USV and juvenile USV.

AIM(S): We aimed to investigate mania-like elevated drive by studying effects of the psychostimulant d-amphetamine (AMPH) on locomotor activity.

METHOD(S): To measure isolation-induced pup USV, mice were isolated from the mother and littermates for 10min on postnatal day (PND) 3, 6, 9, and 12. Juvenile reciprocal social interaction was tested on PND 23. Mania-like elevated drive was investigated by treating mice with 2.5mg/kg AMPH.

RESULTS: In addition to increased call rate, mouse pups lacking the miR379-410 cluster displayed increased peak amplitude and frequency modulation. Juvenile knockout pairs spent significantly more time interacting with each other and emitted more pro-social USV as compared to wildtype pairs. Mutant as well as wildtype mice reacted to AMPH treatment by a significant increase in locomotor activity, and no genotype differences were evident, indicating lack of mania-like behavior in miR379-410 mutants.

CONCLUSIONS: Taken together, the present study confirms and extends previous findings, showing that deletion of the miR379-410 cluster leads to altered communication without affecting psychostimulant-induced hyperactivity.

P7.5. ADULT NEUROGENESIS IN OPOSSUMS AND HIPPOCAMPAL-DEPENDENT LEARNING

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INTRODUCTION: Adult hippocampal neurogenesis occurs in many mammalian species, including the laboratory opossum (*Monodelphis domestica*). Newborn neurons in the dentate gyrus (DG) of the hippocampal formation are involved in learning and spatial memory. The rate of neurogenesis decreases with aging, which was suggested as the cause of the deterioration of cognitive functions.

AIM(S): The aim of the study was to examine association between adult neurogenesis in the DG and spatial memory in young and aged opossums

METHOD(S): To understand whether new neurons contribute to learning and memory, we performed experiments on young and aged laboratory opossums using the Morris water maze test in which animals learn to locate the hidden platform. After behavioral test, sections from opossum brains were immunostained with doublecortin – a marker of newly born neurons – to investigate the rate of adult neurogenesis in the DG.

RESULTS: In the group of young opossums, the time required to find the hidden platform was already significantly lower on the third day of training (*vs.* day 1, $p<0.005$), while in aged opossums a significant difference was observed on the fourth day of training (*vs.* day 1, $p<0.02$). The level of neurogenesis in the DG of the hippocampal formation was lower in the aged opossums than in the young animals.

CONCLUSIONS: However, even the low number of newly formed neurons in the DG of aged opossums are likely to be involved in the formation of spatial memory.

FINANCIAL SUPPORT: This research was supported by the National Science Centre Poland, grant number 2015/17/B/NZ4/02410.

P7.6. FEAR CONDITIONING AFFECTS REACTION TO ULTRASONIC SIGNALS IN SHR AND WISTAR RATS

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INTRODUCTION: Ultrasonic vocalizations (USV) are means of communication between rats. We are studying them by presenting USV or artificial tones from a speaker (playback experiments) and observing vocal (rat's own USV), behavioral, and cardiovascular (heart rate, HR) reactions. We used Wistar rats, which are common in USV experiments, and SHR (spontaneously hypertensive rats), whose USV habits have not been investigated.

AIM(S): We are especially interested in the role of the autonomic nervous system. Therefore, we are investigating the impact of fear conditioning which affects autonomic balance in Wistar rats and SHR with higher activity of the sympathetic system.

METHOD(S): Three different protocols were used (1x, 6x, or 10x; 1 mA, 1 s shocks) and later, the animals were presented with 50 kHz (appetitive) or 22 kHz (aversive) USV. On the day of conditioning, Wistar rats emitted

22 kHz USV immediately after the first electric impulse, while SHR remained silent typically to the sixth-eight shock. Levels of freezing were similar in both strains. On the following day, during ultrasonic signals presentation, after 50 kHz USV playback, SHR did not show a rise in HR nor an increase in their own USV emission, which were both observed in Wistar rats. Both strains responded to 22 kHz USV by a decrease in HR, independently of fear protocol. During the conditioning test, the day after playback experiments, Wistar rats showed lower HR following 1x conditioning. Also, a dramatic rise in numbers of USV was observed in some of 6x animals. Only the HR of 1x conditioned Wistar rats was lower than in the control (not conditioned) group (HR of 6x and 10x Wistar rats did not differ from control group), while in SHR, all conditioned groups tended to have higher HR than controls.

CONCLUSIONS: We confirm that fear conditioning affects the reaction to ultrasonic signals in SHR and Wistar rats. Presumably the autonomic nervous system participates in reactions to USV playback; however, further research with pharmacological agents is essential. To our best knowledge, these are the first studies about USV in SHR.

FINANCIAL SUPPORT: This work was supported by National Science Centre, Poland, grant no. 2015/19/B/NZ4/03393.

P7.7. MOTOR INFORMATION INCREASES VISUAL AWARENESS RATINGS: A TMS-MEP STUDY

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INTRODUCTION: This study concerns the influence of non-perceptual information on visual awareness. We focus on the contribution of action evidence to visual awareness ratings using single-pulse Transcranial Magnetic Stimulation (sp-TMS). This study aims to inform the current debate on the cognitive and neural mechanisms underlying awareness.

AIM(S): The first aim of the study was to investigate the influence of TMS-induced motor response on awareness ratings. The second aim was to establish whether TMS-induced motor evoked potentials (MEPs) measure the amount of accumulated evidence for a certain discrimination task response and differentiate the level of visual stimulus awareness.

METHOD(S): By employing a stimuli identification task, we measured to what extent participants are sensitive to visual information and, using the Perceptual Awareness Scale (PAS), we collected participants' subjective ratings of their visual experience of stimulus. In a within-subject design involving 46 volunteers, the tasks were coupled with sp-TMS and electromyography to manipulate participants' stimulus awareness assessment and record MEP.

RESULTS: We observed higher PAS ratings in the primary motor cortex (M1) sp-TMS condition than in the control condition, but only for responses congruent with the sp-TMS. Identification response reaction times (RTs) in these conditions were higher than in the control condition. The MEP amplitudes increased together with the PAS ratings in sp-TMS congruent responses as compared to incongruent. We also observed that the sp-TMS condition is accompanied by prolonged RTs in the identification task.

CONCLUSIONS: Motor response can be conceived as a factor that influences awareness. We also argue that MEPs might serve as an indirect measure to predict both perceptual and non-perceptual evidence accumulated to visual awareness ratings. Finally, we conclude that the integration of additional information that affects awareness ratings seems to require additional time, thus it was accompanied by prolonged RTs in the identification task. Our results suggest that stimulus-related motor activity influences visual awareness, extending the classical view on how visual awareness is shaped.

P7.8. INTRA VENTRAL TEGMENTAL AREA NORADRENERGIC RECEPTOR SIGNALLING REGULATES CUE-INDUCED FEAR MEMORY RETRIEVAL

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INTRODUCTION: Noradrenergic receptors (α-AR) in the ventral tegmental area (VTA) regulate phasic dopamine release in the mesolimbic, but not in the mesocortical pathway. Accordingly, the α-ARs modulate conditioned behaviours, such as acquisition of fear memories. Importantly, the role of the VTA noradrenergic signaling in fear memory retrieval remain unclear.

AIM(S): The aim of the study was to investigate the role of α_1-AR and α_2-AR in VTA in the retrieval of conditioned stimulus (CS)-induced fear memories. In control studies, we investigate their role in the locomotor activity and anxiety-like behaviour.

METHOD(S): We performed fear conditioning and open field tests in adult male, Spraque-Dawley rats com-

bined with intra-VTA microinfusions. The fear conditioning consisted of two phases: training during which CS was associated with footshock, and retrieval of the conditioned fear memory. Retrieval test was preceded by intra-VTA administration of α_1-AR and α_2-AR antagonist (terazosin 1 µg/side and RX 821002 2.7 µg/side) and conducted in a novel context in which presentation of CS induced fear responses.

RESULTS: We demonstrated that α_1-AR blockade in the VTA decreased freezing responses during retrieval of CS-induced fear memory. In contrast, α_2AR blockade had no effects. Furthermore, neither blockade of α_1-AR and α_2-AR had no effects on locomotor activity and anxiety-like behavior.

CONCLUSIONS: Here, we demonstrated a potential role of the VTA noradrenergic signaling in fear memory retrieval. These results indicate that the noradrenergic signalling in the VTA modulates CS-fear induced responses predominantly via α_1-AR.

P7.9. 5-HT7 RECEPTORS ON GABAERGIC NEURONS MODULATE THE INHIBITORY TONE TO PRINCIPAL CELLS IN THE MOUSE BASAL AMYGDALA

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INTRODUCTION: The amygdala mediates unconscious reactions and is responsible for emotional memory formation and attachment of subjective emotional valence to various stimuli. The amygdala complex expresses 5-HT_7 receptors in a high density, however, their function in this structure remains poorly investigated.

AIM(S): The present experiments were aimed at determining the effects of 5-HT7 receptor activation on membrane properties and synaptic transmission in pyramidal-like basal amygdala (BA) neurons.

METHOD(S): Whole-cell patch clamp recordings were performed on the brain slices containing a part of the amygdala. Spontaneous excitatory and miniature postsynaptic currents (sEPSCs and mEPSCs) were recorded at a holding potential of -70 mV. Spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs) were recorded at a holding potential of 0 mV with pipette filled with cesium gluconate-containing solution.

RESULTS: Activation of 5-HT_7 receptors decreased the mean frequency of sEPSCs without changing sEPSCs amplitude. The mean frequency and amplitude of sIPSCs were enhanced after 5-HT_7 receptor activation. Administration of 5-HT_7 receptors agonist 5-CT induced a hyperpolarization and an increase of the membrane resistance in a majority of recorded cells. The frequency and amplitude of mEPSCs and mIPSCs were not changed after 5-CT administration. The observed effects of 5-HT_7 receptors activation were absent in the presence of the 5-HT_7 receptor antagonist SB 269970. The application of 5-CT had no effect in slices prepared from 5-HT_7 knockout mice.

CONCLUSIONS: These data suggest that the observed decrease in sEPSCs and an increase in sIPSCs frequency and amplitude result from activation of 5-HT_7 receptors located on GABAergic interneurons that, in turn, innervate BA projection neurons.

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P7.10. UP-REGULATION OF PI3K-AKT-MTOR SIGNALING PATHWAY IN NEURONS AFFECTS COGNITIVE FUNCTIONS AND SOCIAL INTERACTIONS IN A MOUSE MODEL

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INTRODUCTION: The PI3K-Akt-mTOR pathway plays an important role in neuronal plasticity. In normal conditions, activity of this pathway is controlled by Pten phosphatase.

AIM(S): We showed that loss of Pten gene in neurons evoked long-term up-regulation of PI3K-Akt-mTOR and temporarily improved learning and memory in mouse models. Moreover, we observed changes in mice vocalization during social interaction and in cellular physiology during electrophysiological recordings.

METHOD(S): Mice model: Inactivation of Pten gene was investigated in 2 models: Pten/CaMKCreERT2, and Pten-flox injected by AAV vectors. The mutation was restricted to forebrain and hippocampal neurons, respectively. Behavioral testing: Both models and respective controls were tested in a learning and memory test in IntelliCage. We measured spatial learning with appetitive behaviors. We also measured the ability to associate

an aversive stimulus in the Contextual Fear Conditioning and social interaction in the Three Chamber Sociability and Social Novelty. Life span: Long-term activity of the PI3K-Akt-mTOR pathway led to increased mortality of Pten/CaMKCreERT2 mutants.

RESULTS: IntelliCage: We discovered better performance of Pten/CaMKCreERT2 mutants in the PL task. The memory improvement lasted to even 24 hours before the death. FC task: Mice developed stronger aversive memory than controls, manifested as increased freezing behavior. Both mutant models showed improved cognitive functions, and Pten/CaMKCreERT2 mice showed a decrease life span.

CONCLUSIONS: Pten-flox-AAV mice developed enhanced contextual fear memory before neurodegeneration in hippocampus occurred and Pten-flox-AAV mice had intensified vocalizations with disturbed sound architecture in social interactions

P8.1. METHYL-CPG BINDING DOMAIN 3 PROMOTER ACTIVITY IN A RAT MODEL OF SEIZURE EVOKED BY INTRAPERITIONAL INJECTION OF PENTYLENETETRAZOL (PTZ)

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INTRODUCTION: Animal models for seizures and epilepsy have played a fundamental role in advancing our understanding of basic mechanisms underlying epileptogenesis and epilepsy. During epileptogenesis and epilepsy, several molecular and cellular changes occur, including alterations in gene and protein expression. MBD3 (Methyl-CpG binding domain 3) protein is a reader of DNA methylation marks, which changed its expression in epileptogenesis.

AIM(S): The aim of this study was to determine changes in MBD3 protein expression after acute seizure in the rat brain.

METHOD(S): Spraque-Dawley rats were kept in an enriched environment and were subjected to handling procedure. A single interperitional injection of pentylenetetrazol (PTZ, 40 mg/kg) was used to evoke tonic-clonic seizure. Control rats (n=16) which were injected by saline and rats after PTZ administration (n=16) were observed for an hour after injection. To examine changes in RNA expression and protein level, animals were sacrificed at selected time points: 1 h, 4 h, 8 h and 24 h after injection. Changes in MBD3 protein levels were examined in the hippocampus, entorhinal, and somatosensory cortex using Western Blot with anti-MBD3 antibody (#A302-528A, Bethyl) followed by ImageJ analysis, whereas changes on RNA level were examined with Real Time PCR.

RESULTS: No significant differences were observed in RNA levels in the hippocampus, entorhinal, and somatosensory cortex during 24 h after injection. Western Blot analysis showed an increased level of MBD3 protein at 4 h after seizures evoked by PTZ injection in the somatosensory cortex. PTZ did not affect MBD3 protein expression in the hippocampus and entorhinal cortex at 4 h, 8 h, and 24 h after injection, nor in the somatosensory cortex at 8 h and 24 h after PTZ injection.

CONCLUSIONS: These results showed that seizures influence MBD3 protein expression and therefore MBD3 may play an important role in epileptogenesis or epilepsy.

FINANCIAL SUPPORT: This work is supported by the Polish Ministry of Science and Education grant 2015/19/B/NZ4/01401.

P8.2. HYPEROSIDE ISOLATED FROM IMPATIENS GLANDULIFERA ROYLE ALLEVIATES DEPRESSIVE AND ANXIETY-LIKE RESPONSES IN A MOUSE MODEL OF POSTTRAUMATIC STRESS DISORDER

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INTRODUCTION: Posttraumatic stress disorder (PTSD) is a chronic and prevalent psychiatric condition that may develop following exposure to traumatic events. Depressive symptoms and anxiety belong to the most frequent symptoms observed in PTSD patients. Less than 30% of PTSD patients achieve full remission with the use of available drugs. For this purpose, there is a clear need to develop more efficient and safer drugs as alternative and/or complimentary therapy for PTSD. Hyperoside (HYP) is one of the polyphenols found in *Impatiens glandulifera*. Our previous experiments showed that HYP exerted antidepressant effects, both after acute and chronic (14 days) treatment in mice in the forced swimming test (FST; data not published).

AIM(S): The present study aimed to investigate the effect of HYP on the behavioural impairments (depression and anxiety) induced by a mouse single prolonged stress (mSPS) – a rodent model of PTSD.

METHOD(S): mSPS protocol: mice were exposed to a series of short stressors. In particular, they were restrained for 2 h in a Plexiglas tubes (50 ml), placed in glass beakers and immersed in water (23-25°C) for a group swim (10 min). Then, they were exposed to a beaker of soiled bedding taken from cages of rats (15 min), and at the end, they were exposed to anhydrous diethyl ether until they lost consciousness (ap-

prox. 2 – 3 min). Seven days after exposure to SPS, the administration of substances was started (during next 14 days). Then, animals were subjected to behavioural tests, including the elevated plus-maze test (EPM), measurement of locomotion, and FST.

RESULTS: Mice given chronically HYP (3.75 and 7.5 mg/kg) after exposure to mSPS exhibited a reduction of immobility time in FST, and more open arm entries and longer open arms duration in EPM without affecting locomotor activity as compared to control-mSPS group.

CONCLUSIONS: In summary, our results suggested the potential of HYP in alleviating the mSPS-induced depressive and anxiety-like responses.

P8.3. MECHANISM OF MMP-9-1562C/T SINGLE NUCLEOTIDE POLYMORPHISM-DEPENDENT REGULATION OF MMP-9 (MATRIX METALOPROTEINASE-9) EXPRESSION IN HUMAN NEURONS

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INTRODUCTION: MMP-9-1562C/T modulates MMP-9 mRNA expression and consequently influences the course of many human diseases that involve pathology of this metalloproteinase (e.g., stroke, Alzheimer's disease, Parkinson's disease, multiple sclerosis, cardiovascular diseases). Until now, the precise molecular mechanism of MMP-9-1562C/T-dependent influence on MMP-9 gene expression has not been discovered.

AIM(S): The purpose of this study is to identify transcriptional regulators binding to MMP-9-1562C/T and to evaluate their influence on MMP-9 expression in human neurons.

METHOD(S): The studies are carried out in differentiated neurons derived from the SH-SY5Y human neuroblastoma cell line. We showed, by luciferase assay, that transcriptional activity of the T allele is higher than the C allele in human neurons. We also studied interactions of nuclear proteins with MMP-9-1562C/T polymorphism by EMSA (Electrophoretic Mobility Shift Assay). We found that nucleoprotein complexes form in an allele-specific manner in human neurons. Using magnetic beads coated with the human allele C or T, we pulled down nuclear proteins binding specifically to the alleles. Then, we analyzed the identity of these proteins using mass spectrometry.

CONCLUSIONS: As a result, we identified numerous transcriptional regulators and co-regulators that may be involved in the allele-specific modulation of MMP-9 expression in human neurons.

P8.4. LOCATION OF HUR PROTEIN IN A MODEL OF PHOTOTHROMBOTIC ISCHEMIC STROKE IN RAT – METHOD DEVELOPMENT

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INTRODUCTION: Cerebrovascular diseases are the principal causes of mortality and disability worldwide. Creation of a repeatable, very precise, and minimally invasive animal model of ischemic stroke is necessary. A well-designed model will facilitate future research on new possibilities of treatment of ischemic stroke. The penumbra is the area surrounding necrosis in ischemic stroke. It is a strategic area for therapy. Survival and restoration of correct circulation is the goal of treatment of ischemic stroke. Protein HuR is a member of the ELAVL protein family. This protein has one of the best-known functions in stabilizing mRNAs in order to regulate gene expression.

AIM(S): The first aim of our research was to develop, test, and select the most favorable parameters in a model of photothrombotic ischemic stroke in rat. The second goal was to determine the location of the HuR protein during an ischemic stroke, especially in the penumbra.

METHOD(S): Phototrombotic ischemia was produced in 54 male Long-Evans rats by evoking the clotting of blood inside the cerebral vessels of the rat, through activation of Bengali rose with the light of 3200 K energy. Bengal rose was given by a tail vein. We tested different exposure times for light (10 min/ 15 min/ 20 min) and different life times of rats from surgery to euthanasia (6 h, 12 h, 24 h, 48 h, 7 days, and 14 days).

RESULTS: We performed immunohistochemistry (IHC) tests on brain tissue after ischemic stroke. After analyzing the results, we obtained the best parameters: 15 min exposure to light and life time (12 h – 48 h). These parameters made it possible to obtain a small necrosis area and a large penumbra area. We observed increased concentration of protein HuR in the cytoplasm of panumbra neurons between 12 h – 48 h after stroke.

CONCLUSIONS: Our model of small cortical ischemic stroke is repeatable, very precisely located, and low invasive. During ischemic stroke, there is increased transport of HuR proteins to the cytoplasm of neurons, especially in the penumbra as compared to healthy tissue.

P8.5. PUPS' ALTERED ULTRASONIC VOCALISATION IN THE POLY I:C RAT MODEL OF AUTISM SPECTRUM DISORDER: RESULTS FROM THE MOTHER ISOLATION TEST

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INTRODUCTION: A large body of evidence suggests a connection between maternal infection during pregnancy and increased risk of developing autism spectrum disorder (ASD) in the child. One of the characteristic symptoms of ASD is deficits in communication. Rodent models of ASD include the administration of a synthetic double-stranded RNA, the polyinosinic-polycytidylic acid (poly(I:C)) to the pregnant dam, that evokes an antiviral-like immune reaction. Rat pups isolated from their mother emit calls within ultrasonic spectrum of ~40 kHz.

AIM(S): In this study, we examined whether poly I:C pups presented an altered pattern of ultrasonic vocalization (USV) during the mother isolation test.

METHOD(S): Pregnant Sprague-Dawley dams received an intraperitoneal injection of poly I:C (5 mg/kg) or vehicle on GD 15. The isolation of male and female offspring was performed on PND 6.

RESULTS: We observed changes in the number of vocalisations and an altered structure of emitted calls. Poly I:C males emitted less calls than control animals. A similar change in females was not observed. Both male and female poly I:C pups emitted calls of lower call bandwidth and peak frequency.

CONCLUSIONS: Such changes of the structure of emitted calls suggest an impairment of vocal communication in the poly I:C animals. A decrease in the number of emitted calls in poly I:C males may reflect the fact that the prevalence and severity of symptoms of ASD is higher in boys and it appears that this higher susceptibility of males is present also in the poly I:C model.

FINANCIAL SUPPORT: This study was supported by the Polish National Science Centre grant NCN 2016/23/B/NZ7/01131.

P8.6. NMDA RECEPTOR MODULATION OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS DECREASES THE NUMBER OF TYROSINE HYDROXYLASE POSITIVE CELLS IN THE VENTRAL TEGMENTAL AREA AND SUBSTANTIA NIGRA PARS COMPACTA IN RATS

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INTRODUCTION: Two notable targets of the pedunculopontine tegmental nucleus (PPN) circuitry, the substantia nigra (SN) and the ventral tegmental area (VTA), are implicated in locomotion and reward processing. A dysfunction of these regions occurs in Parkinson's and related disorders as well as in various psychiatric conditions, and over the course of normal aging.

AIM(S): In the present study, we were interested in understanding NMDA-receptors involvement in the interactions between the PPN and SN/VTA midbrain complex. In order to obtain more insight into this process, we analyzed the number and the distribution of midbrain tyrosine hydroxylase positive cells (TH+).

METHOD(S): All rats were implanted with bilateral stimulating electrodes in the VTA and with bilateral guide cannulas for intracerebral injections into the PPN. Immunohistochemistry for TH+ was used to measure the number of active dopaminergic neurons in midbrain (VTA-SN) of rats subjected to unilateral VTA electrical stimulation and local injection of MK-801 (5 μg) or NMDA (3 μg) to the contralateral or ipsilateral hemispheres into the PPN (4 experimental groups). The control brains were from rats in which only the 14-day unilateral electrical VTA-stimulation was performed (control group).

RESULTS: Immunohistochemical analysis revealed a decrease in the number of TH+ cells in the midbrain. When the main subdivisions of the VTA/SN were subjected to a separate analysis, a significantly lower number of TH+ cells were found in all experimental groups in the PBP (parabrachial pigmented nucleus), PB (paranigral nucleus) and SNc (SN, pars compacta), as compared to the control group.

CONCLUSIONS: The level of NMDA receptor arousal in the PPN regulates the activity of the midbrain dopaminergic cells.

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P8.7. LET GENES TALK: TRANSCRIPTOMIC CHANGES IN THE PFC INDUCED BY L-DOPA IN HEMIPARKINSONIAN RATS

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INTRODUCTION: The hallmark symptoms of Parkinson's disease (PD) are progressive motor impairments. Nevertheless, PD is also associated with altered executive function and other cognitive impairments. While treatments of PD provide at least temporary relief from the motor symptoms, the effects of L-DOPA on the cognitive impairments may provide mixed effects and require further investigation.

AIM(S): Here we assess changes in gene expression in the prefrontal cortex (PFC) of rats with unilateral lesion of midbrain dopamine neurons.

METHOD(S): Male Wistar Han rats were infused with 6-hydroxydopamine (6-OHDA, 8 µg/4 µl) into the left medial forebrain bundle. The experimental animals were treated i.p. with L-DOPA (12.5 mg/kg) supplemented with benserazide hydrochloride (6.25 mg/kg) daily for 14 days. An hour after the last dose, the rats were killed, and the left and right PFC were isolated separately. Analysis of gene expression was performed by RNA-seq (Illumina PE 150, 20M pair reads per sample). Reads were aligned to rn6 rat reference genome using hisat2 2.1.0.

RESULTS: We identified 12,459 genes with FPKM > 1 after L-DOPA treatment in both ipsi- and contralateral portions of the PFC of rats lesioned with 6-OHDA. Two-way ANOVA revealed 48 genes with differential expression profiles. The effect of treatment was the most pronounced, and included transcripts linked to activity-regulated expression in neurons and metabolism in the glia. Ontology analysis of the genes with altered expression indicated over-representation of terms associated with cytokine and glucocorticoid signalling. The involvement of altered glucocorticoid signalling induced by L-DOPA treatment was also confirmed by analysis of the promoter regions of the regulated genes.

CONCLUSIONS: Unilateral lesions of dopamine neurons lead to enhanced sensitization of neurons in PFC to L-DOPA action. We show that, to a large extent, these changes appear to bilaterally affect the molecular profile of PFC.

P8.8. EBI2 KNOCK-OUT MICE SHOW GREATER LOSS OF BRAIN LIPIDS FOLLOWED BY EARLIER ATTEMPTS AT REMYELINATION IN THE CUPRIZONE MODEL OF DEMYELINATION

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INTRODUCTION: The EBI2 receptor is one of the key mediators of innate immune responses. In cooperation with its ligand, oxysterol 7α,25HC, EBI2 coordinates immune cell positioning in the secondary lymphoid tissue, enabling appropriate humoral and cellular immune responses. EBI2 is also expressed in the central nervous system (CNS), where it regulates inflammatory signalling and myelination. Importantly, EBI2 receptor's altered expression and signalling have been linked to a range of diseases including multiple sclerosis.

AIM(S): The aim of the study was to investigate the effects of EBI2 signalling on lipid parameters in the cuprizone model of demyelination.

METHOD(S): 37-week-old EBI2 knock-out (KO) and wild-type C57BL6J mice were fed a 0.2% cuprizone diet for 5 weeks. The animals were decapitated immediately after 5 weeks or after an additional 2 weeks of recovery period on normal diet. Here, we report greater loss of brain cholesterol and triglycerides in EBI2 KO mice after 5 weeks on the cuprizone diet, indicating EBI2 receptor involvement in CNS lipid maintenance under demyelinating conditions.

RESULTS: However, two weeks after return to normal diet, when spontaneous remyelination is observed, the data showed higher cholesterol and triglycerides levels and a greater increase in lipase activity in the EBI2 KO mice. Other studies showed that a sharp increase in lipase activity is observed in an experimental autoimmune encephalomyelitis model around the time of symptom remission and in cerebellar slices between de- and re-myelination phases.

CONCLUSIONS: Our data is, therefore, in line with these findings showing that earlier lipase activity in the EBI2 KO mice possibly leads to an earlier remyelination attempt, as observed by increased cholesterol and triglycerides levels. These results indicate functional involvement of EBI2 receptor in lipid homeostasis under pathophysiological conditions and thus warrant further investigations into the role of EBI2 in demyelinating diseases.

P8.9. THE POTENT ROLE OF CATECHOLAMINERGIC INNERVATION IN LOCOMOTOR RECOVERY INDUCED BY INTRASPINAL GRAFTING OF EMBRYONIC BRAINSTEM TISSUE IN ADULT PARAPLEGIC RATS

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INTRODUCTION: In mammals, spinal cord transection results in permanent loss of locomotor function. Our previous investigations demonstrated that intraspinal grafting of an embryonic (E14) brainstem raphe area enhances hindlimb locomotor recovery in adult paraplegic rats. This process is mediated mainly through serotonergic (5-HT) neurons.

AIM(S): The aim of the present investigation was to determine the role of catecholaminergic (CA) neurons that are present in the grafted tissue in this recovery.

METHOD(S): The experiments were performed on the inbred strain WAG rats after spinal cord total transec-

tion. Grafts were placed in the spinal cord below the total transection and included 5-HT neurons derived from the embryonic (E14) brainstem. Two months later, locomotor performance was tested with chronic EMG recordings from Soleus (Sol) and Tibialis Anterior (TA) muscles, allowing quantification of limb movement recovery. After completing the functional testing, the spinal cords were harvested for morphological investigations.

RESULTS: In the graft area, besides 5-HT neurons, we found a number of CA neurons of graft origin. The CA innervation, however, was weaker than that of 5-HT and limited to specific areas of the spinal cord. We showed that the 5-HT and CA neurons were fully differentiated at the time of tissue dissection for grafting and that the host environment did not stimulate their proliferation and differentiation. The locomotor abilities of the spinal grafted rats were facilitated by application of Yohimbine and suppressed by Clonidine, likely through their actions on noradrenergic autoreceptors.

CONCLUSIONS: Our results indicate a potent role of the CA innervation in locomotor recovery in paraplegic rats. In addition to the important role of 5-HT neurons in this process, our findings provide new insights into the mechanisms underlying the locomotor recovery in rats.

FINANCIAL SUPPORT: This work was supported by ERA-NET NEURON CoFund Consortium NEURONICHE (ERA-NET-Neuron/16/17).

P8.10. IMPACT OF KETOGENIC DIET ON GAIT IN PURKINJE CELL SPECIFIC TRANSGENIC MOUSE MODEL OF TUBEROUS SCLEROSIS COMPLEX

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INTRODUCTION: The ketogenic diet is a high fat low carbohydrate diet, wherein the majority of caloric needs is covered by fats with very low carbohydrate intake. The diet is widely used not only by athletes and patients suffering from obesity or diabetes, but also by patients with intractable epilepsy. The high fat, low carbohydrate diet is extensively studied within the fields of numerous diseases including cancer and neurological disorders.

METHOD(S): In present study, we used Purkinje cell (PCs) specific knockout mice lacking hamartin (tsc1), a key protein involved in mTORC1 pathway. Deletion of Tsc1 gene in PCs results in a loss of these cells and gait impairments. We implemented a ketogenic rodent chow to reveal its potential influence on prevention of loss of PCs in the cerebellum. We assessed the effects of treatment with the ketogenic diet on the quality of mice gait. The gait was analysed in the CatWalk system from Noldus. Obtained data were compared among groups: control animals, with tsc1 in PCs, fed with standard rodent diet, animals with tsc1 but fed with ketogenic chow and knockout mice, fed respectively with two types of abovementioned diets.

RESULTS: Our results revealed that, as expected, animals without hamartin in PCs present severe gait disturbances. Supplementation of the ketogenic diet has no effect on gait disturbances caused by deletion of tsc1 in PCs.

CONCLUSIONS: Additionally, statistical analysis of data obtained from animals without gene deletion didn't bring any proof of differences in gait parameters between groups fed with two different chows.

P8.11. IMPACT OF ENVIRONMENTAL ENRICHMENT ON ANXIETY AND LEARNING IN THE RAT MODEL OF EPILEPSY INDUCED BY ELECTRICAL STIMULATION OF THE AMYGDALA

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INTRODUCTION: The environment plays an influential role in the development of many brain disorders; however, its role in modulation of the epilepsy phenotype has not been studied in detail.

AIM(S): The aim of this study was to investigate whether environmental enrichment impacts anxiety and learning in an experimental model of epilepsy.

METHOD(S): Male Sprague-Dawley rats were allocated to either environmentally enriched (EE; n=13) or standard housing conditions (SH; n=13). Epilepsy was induced by SE (Status epilepticus) evoked by electrical stimulation of the amygdala (25 min, 100 ms train of 1 ms, 60 Hz bipolar pulses, 400 μA, every 0.5 s). The following tests were conducted to assess the behavior of animals: behavioral hyperexcitability, open field, new object recognition, elevated plus maze, social interactions, and the Morris water maze. Blood was withdrawn on days 7 and 29 after stimulation and on the day of perfusion, to assess cortisol levels.

RESULTS: Environmental enrichment significantly reduced anxiety levels. We observed reduced mobility in the open field test, a decrease in total distance traveled in the social interactions test, and decreased touch-response in the behavioral hyperexcitability test. SH animals showed impaired spatial memory and learning as compared to EE animals. Rats from the EE group spent more time near a platform in the Morris Water Maze test. Moreover, in RODA analysis, EE control animals showed a trend towards lower thigmotaxis

compared to SH animals starting from the 2nd day trial 2, with a significant difference obtained in the 3rd day. Blood analysis demonstrated that SH rats had a significantly higher level of cortisol compared to EE rats.

CONCLUSIONS: The present study indicates that environmental enrichment had beneficial effects on anxiety and learning and memory, which may be caused by lower stress hormone levels.

FINANCIAL SUPPORT: This work was supported by the FP7-HEALTH project 602102 (EPITARGET) and Polish Ministry of Science and Education grant W19/7. PR/2014.

P8.12. CEREBRAL ADMINISTRATION OF ALPHA-SYNUCLEIN MODULATES INFLAMMATORY REACTION IN THE NIGRO-STRIATAL SYSTEM: A COMPARISON OF MALES AND FEMALES

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INTRODUCTION: The risk of developing Parkinson's disease (PD) is twice as high among men as among women. Estrogens appear to be a protective factor. The progression of PD is characterized by inflammation, especially the activation of the microglia. The data suggests that increased levels of α-synuclein (ASN) can induce microglia activation. Activated microglial cells release pro- and anti-inflammatory cytokines.

AIM(S): The aim of this study was to investigate the role of increased ASN monomers concentration as a major pathogenic factor causing microglia response and changes in the expression of mRNA of inflammatory cytokines (i.e., interleukin 1α (IL- α), IL-10, IL-12 and tumor necrosis factor α (TNFα)) in the striatum (ST). We also examined the levels of ionized calcium binding adaptor molecule 1 (Iba-1). We evaluated the differences between the genders after injection of ASN.

METHOD(S): Male and female C57Bl/10 Tar 9-month-old mice were used in this study. Human recombinant ASN was bilaterally administered into ST (single treatment – 4 μg/structure, 8 μg per brain) and mice were decapitated after 4-or 12-weeks post injection. Changes in the level of inflammatory factors in ST were evaluated using Real-Time PCR.

RESULTS: We observed increased levels of a microglia marker, Iba1 protein, in all experimental groups after 4 weeks of injection of ASN into the ST, with the highest differences in the FOVA group (female after ovariectomy). IL-10 mRNA levels were significantly elevated in the FOVA group at 4 weeks after ASN administration into the ST as compared to the ovariectomy control group. We noticed differences in levels of IL-1α, IL-12, and TNFα mRNA between the genders after injection of ASN.

CONCLUSIONS: Our results support the hypothesis of pro-inflammatory actions of ASN monomers. Injection of ASN into the ST induces microglia activation. Estrogens appear to be an important factor in the inflammatory reaction in the murine model of Parkinson's disease after cerebral administration of ASN.

P8.13. HFO UNDER KETAMINE-XYLAZINE ANESTHESIA ARE COUPLED TO LARGE CURRENT SOURCES AND NASAL RESPIRATION: A PROPOSED MECHANISM FOR THE GENERATION OF HFO AFTER NMDAR BLOCKADE

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INTRODUCTION: Over the past decade, we and other groups have shown that ketamine and other NMDA receptor antagonists evoke high-frequency oscillations (HFO; 130-180 Hz) in a variety of rodent cortical and subcortical regions.

AIM(S): Our recent studies show that the olfactory bulb (OB) appears to be particularly important for the generation of this activity. To date, this activity has mainly been recorded in awake rats; however, there is some evidence that fast oscillation can be recorded in the OB of rodents under ketamine xylazine anesthesia.

METHOD(S): LFPs in the OB were recorded using twisted stainless-steel electrodes in rats under ketamine 100 mg/kg + xylazine 10 mg/kg anesthesia (KX) or a subanesthetic dose of ketamine 20 mg/kg. In a second study, rats were implanted with thermocouples for simultaneous recording of nasal respiration and LFPs in the OB. In a third study, 32 channel silicon probes were used to record LFPs under KX. KX was associated with the emergence of a fast oscillations, around 120 Hz (which we termed KX-HFO) that occurred in bursts nested on slower oscillations. This is similar to HFO that occurs in awake rats following subanesthetic doses of ketamine. KX-HFO were attenuated by unilateral naris blockade and reversed phase close to the mitral layer – also similar to the awake state.

RESULTS: Simultaneous recordings from the nasal cavity (with thermocouples) and LFPs showed that KX-HFO was tightly coupled to nasal respiration, around 2 Hz. Spatial profile of LFPs recorded across the OB revealed strong HFO current sources close to the mitral layer that was preceded by a large current sink (around 2 Hz) more ventrally (in the extraplexiform/glomerular layers).

CONCLUSIONS: Nasal respiration drives afferent input to the OB that produces corresponding large current sinks (local depolarization) in the OB which under KX anesthesia (and more generally NMDAR blockade) leads to the emergence of HFO by stimulating mitral/tufted neurons at their apical dendrites.

P8.14. PRENATAL STRESS-INDUCED SEX DIFFERENCES IN THE INCIDENCE AND THE COURSE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) IN ADULT RATS: OFFSPRING OF MOTHERS WITH DIFFERENT SENSITIVITY TO EAE

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INTRODUCTION: Previous studies have revealed that factors affecting the incidence and the course of multiple sclerosis and EAE are genetic predisposition, sex, sex hormone levels, and activity of the hypothalamic-pituitary-adrenal axis (HPA). Chronic stress experienced by pregnant mothers leads to changes in sex hormones levels and HPA axis activity in adult offspring.

AIM(S): This research is aimed at investigating the effects of prenatal stress (PS) on the incidence and the course of EAE as well as the level of serum corticosterone (Cort) and annexin A1 (ANXA1) in 200-days-old rats, offspring of females with different sensitivity to EAE induction.

METHOD(S): The incidence and the score of EAE severity were analyzed. Serum Cort and ANXA1 levels were measured before and on the 10th day after immunization. EAE-immunized females were divided into two maternal groups: EAE-sensitive and EAE-resistant. The mothers mated two months after immunization. PS was induced from the 15th day of pregnancy and until the birth under round-the-clock illumination and periodically recurring hypokinesia in pregnant rats. EAE was modelled in all descendants by inoculating a homogenate of rat spinal cord tissue with complete Freund's adjuvant.

RESULTS: The incidence rate in adult rats surviving PS was significantly lower than that in the control group. In the descendants of mothers resistant to EAE immunization, EAE severity rates and the plasma level of ANXA1 collected on day 10 after immunization were significantly lower in prenatally stressed females in comparison with both prenatally stressed males and females with normal embryonic development. The level of Cort collected on day 10 after immunization did not vary.

CONCLUSIONS: The incidence and the course of EAE in rats depends on a combination of factors such as gender, PS, and the EAE-sensitivity of mothers.

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P8.15. NEUROPROTECTIVE PROPERTIES OF CYSTAMINE IN MPTP-INDUCED MURINE MODEL OF PARKINSON'S DISEASE

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INTRODUCTION: Parkinson's disease (PD) is a common neurodegenerative movement disorder. It is characterized by a progressive degeneration of dopaminergic substantia nigra neurons projecting to the striatum, as well as, by an accumulation of intraneuronal Lewy bodies containing misfolded a-synuclein. Neurodegeneration is coincident with a decrease in dopamine, the dopamine transporter, and the dopamine metabolites levels in PD brain. Current therapeutic approaches for the treatment of PD are only symptomatic and do not block neuronal loss. Recently, an enormous amount of work has been conducted to identify molecules that could be used as neuroprotective agents. One of them is cystamine – the inhibitor of transglutaminases activity. Transglutaminases are involved in the formation of a-synuclein aggregates, therefore blocking of its activity may prevent the progression of PD.

AIM(S): The aim of the present study was to examine the effects of cystamine on neurodegenerative processes in the murine model of PD.

METHOD(S): Male 1-year-old C57Bl/10 Tar mice were used in this study. Cystamine was injected intraperitoneally for 14 days, beginning 13 days prior to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication. Changes in the mRNA level of inflammatory factors in striata were examined using Real-Time PCR. Neurotransmitter levels in striata were evaluated by high-performance liquid chromatography (HPLC).

RESULTS: Our study demonstrated that chronic administration of cystamine before MPTP intoxication improved striatal levels of dopamine and its metabolites, as compared to MPTP-treated groups. We also observed an inhibition of inflammatory reaction induced by MPTP (lower expression of microglia marker and proinflammatory interleukin).

CONCLUSIONS: Cystamine exerts anti-inflammatory effects and preserves nigrostriatal function after MPTP intoxication in PD. However, further research must be conducted to provide more evidence of a protective role of cystamine in PD.

P8.16. THE EFFECT OF A SPHINGOSINE--1-PHOSPHATE RECEPTOR MODULATOR ON THE TRANSCRIPTIONAL PROFILE OF GENES LINKED TO GLUTAMATE HOMEOSTASIS IN THE SPORADIC AND GENETIC ANIMAL MODELS OF ALZHEIMER'S DISEASE

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INTRODUCTION: Alzheimer's disease (AD) is neurodegenerative disorder characterized by progressive memory impairment and cognitive failure which leads to dementia in aged population. Lots of data indicate glutamate-mediated neurotoxicity as a one of the pathomechanisms responsible for neuronal cell death during the course of AD.

AIM(S): In this study, we examined the effect of fingolimod (FTY720-modulator of sphingosine-1-phosphate receptors) on the transcription of genes involved in the homeostasis of glutamatergic system in animal models of AD.

METHOD(S): 3- and 12-month-old (3M, 12M) FVB/APP$^+$ transgenic mice with the London (V717I) APP mutation were used in this study. Mice without the mutation (APP$^-$) were used as a control. The sporadic AD model was induced by injection of streptozotocin (STZ, *icv*. 2,5 mg/kg b.w.) in ACSF (vehicle) to 3M C57BL/6 mice. Animals received FTY720 (1mg/kg b.w.) or NaCl (vehicle) for 2 weeks. Brain cortex was isolated and qPCR methods were applied.

RESULTS: Our results indicate a different model-dependent profile of changes in gene expression. We observed significant upregulation of *Slc17a7*(VGluT1), *Grin1*(-GluN1), and *Grm3* (mGluR3) gene expression in APP$^+$ 12M mice. A significant elevation of *Gria1*(GluR-1) and *Grin1* (GluN1) mRNA levels with an accompanying decrease of *Slc17a8* (VGluT3) and *Grm5* (mGluR5) was observed in STZ mice. The administration of FTY720 led to a decrease in *Gria2* (GluR-2) and *Grm3* mRNA levels, as well as, an elevation of *Slc1a3* (GluT-1), *Slc17a7*, and *Slc17a8* mRNA in STZ mice compared to appropriate controls.

Transcriptional changes in vesicular glutamate transporters (*Slc17a7* and *Slc17a8*) as well as glutamate receptors genes (*Grin1*, *Gria1*, *Grm3,5*) suggest that they may be involved in the mechanisms leading to AD.

CONCLUSIONS: FTY720 may potentially modulate the expression of genes involved in homeostasis of the glutamatergic system in the model of sporadic AD.

FINANCIAL SUPPORT: Supported by the National Science Centre grant no. NCN2014/15/B/NZ3/01049.

P8.17. FINGOLIMOD (FTY720 – MODULATOR OF SPHINGOSINE-1-PHOSPHATE RECEPTORS) ALTERS GENES EXPRESSION OF SELECTED NAD+-DEPENDENT ENZYMES IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

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INTRODUCTION: Sirtuins (SIRTs) and poly(ADP-ribose) polymerases (PARPs) belong to the family of NAD$^+$-dependent enzymes. Both are involved in the regulation of energy homeostasis, cellular stress response, and DNA repair. Recent data suggest that alterations of bioactive sphingolipids level as well as SIRTs and PARPs may be involved in Alzheimer's disease (AD) pathology, finally leading to the progression of disease.

AIM(S): In this study, the effect of FTY720 administration on mRNA levels of SIRTs and PARP-1 in an animal model of AD was examined.

METHOD(S): 3-, 6-, and 12-month-old (3M, 6M, 12M) FVB/APP$^+$ transgenic mice with London APP (V717I) mutation were used in this study. Mice without the mutation (APP$^-$) were used as the control. Animals received *i.p.* FTY720 (1mg/kg b.w.) or NaCl (vehicle) for 2 weeks. Brain cortex was isolated and qPCR methods were applied.

RESULTS: A significant downregulation of *Sirt1* mRNA levels in the cortex of 3M APP$^+$ mice *vs.* APP- was observed. We also observed a tendency for a reduction of 6M *Sirt3* and *Sirt4* mRNA levels in APP$^+$ mice. Administration of FTY720 increased mRNA levels of *Sirt1* in 3M APP$^+$ mice as well as *Parp1*, *Sirt1*, *3*, *5* in 6M APP$^+$ mice compared to APP$^+$ mice treated with vehicle. Moreover, FTY720 elevated *Parp1*, *Sirt1*, and *Sirt3* mRNA levels in 12M APP$^+$ mice.

CONCLUSIONS: The results of our study revealed a potential link between bioactive sphingolipids and NAD$^+$-dependent enzymes. These results may also indicate an FTY720-modulatory role in SIRTs and PARPs gene expression and may offer a useful tool in the therapeutic strategy of neurodegenerative disorders. FTY720, through the activation of mitochondrial sirtuins (*Sirt3*,5), may improve anti-oxidative defense and protect cells against oxidative stress evoked by amyloid beta toxicity. Moreover, through activation of *Sirt1* and *Parp1* gene expression, FTY720 may enhance DNA repair processes.

FINANCIAL SUPPORT: Supported by the National Science Centre grant no. NCN 2014/15/B/NZ3/01049 and Mossakowski MRC PAS statutory theme no.7.

P8.18. NASAL RESPIRATION IS CRITICAL FOR KETAMINE-INDUCED HIGH FREQUENCY OSCILLATIONS AND HYPERACTIVITY

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INTRODUCTION: Ketamine, at subanesthetic doses, produces psychotomimetic effects. In rodents, ketamine produces characteristic changes in oscillatory activity that can be recorded in local field potentials (LFP). One effect after systemic injection of ketamine is the emergence of abnormal high frequency oscillations (HFO) 130 -180 Hz that have been described in many rodent brain areas. Recently, we have shown that the olfactory bulb (OB) plays an important role in the generation of HFO after ketamine.

AIM(S): The aim of the present study was to examine the extent to which nasal respiration may drive abnormal HFO after ketamine, in freely-moving rats.

METHOD(S): LFPs (from the OB) and nasal respiration (thermocouples implanted in the nares) were recorded before and after injection (saline or ketamine 20 mg/kg) from male Wistar rats. A separate group of rats was used to study nares blockade. To block the nares, rats were anesthetised and a silicon occluder was inserted into one or both nares. Controls were exposed to initial isoflurane for a comparable amount of time but without blockade. Rats were given recovery and then injected with ketamine.

RESULTS: Ketamine immediately increased exploratory fast sniffing (4-10 Hz), which correlated with increases in locomotor activity and HFO power. Saline injection did not substantially alter these measures. Nasal respiration entrained bursts of ketamine HFO recorded in the OB on a cycle-by-cycle basis. Further, ketamine-induced HFO was attenuated unilaterally by naris blockade on the same side. Bilateral naris blockade reduced power and frequency of HFO and also reduced hyperactivity produced by ketamine.

CONCLUSIONS: Our results suggest that nasal respiration is a powerful drive of HFO after injection of ketamine in the OB. These findings may explain previous observations that ketamine-HFO couples to slower frequencies. Functional nasal respiration appears to be critical for the emergence of both HFO and hyperactivity produced by ketamine.

P8.19. MATERNAL IMMUNE ACTIVATION DURING PREGNANCY ALTERS THE EXPRESSION OF MITOCHONDRIAL DYNAMIC MARKERS IN THE BRAIN OF RAT OFFSPRING

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INTRODUCTION: Prenatal exposure to infection and subsequent inflammatory responses, as well as, mitochondrial dysfunction has been implicated in the pathogenesis of autism spectrum disorders (ASDs). However, the molecular links between infection-induced fetal brain changes, mitochondrial deregulation, and the autistic phenotype remain obscure.

AIM(S): Analysis of maternal immune activation (MIA)-induced changes in the expression of mitochondrial dynamics markers in the brain of the neonatal and adolescent rat offspring.

METHOD(S): The MIA model was induced by single intraperitoneal injection of lipopolysaccharide (100 µg/kg b.w.) to pregnant rats at embryonic day 9.5. On the 7th or 52-53rd post-natal day, rat offspring were decapitated, and the brains isolated. Transmission electron microscopy (TEM), quantitative real-time PCR (qPCR), and immunoblotting were used to determine mitochondrial ultrastructure and mRNA/protein expression, respectively.

RESULTS: The electron microscopic study demonstrated altered mitochondrial morphology, including fragmented cristae, expanded matrix compartment, and membrane disruption in both the cerebral cortex and hippocampus of adolescent MIA offspring. Moreover, changes were noted in the expression of proteins involved in the maintenance of mitochondrial morphology. We observed upregulated fusion machinery proteins – mitofusin 1 (Mfn1), mitofusin 2 (Mfn2), and Opa1 – as well as mitochondrial fission proteins – dynamin related protein-1 (Drp1) and fission protein 1 (Fis1) – in the neonatal MIA brains. However, in adolescent animals exposed to prenatal infection, the expression of Mfn1, Mfn2 and Opa1 was significantly reduced; nevertheless, Drp1 and Fis1 remained increased.

CONCLUSIONS: MIA-evoked perturbations in the proteins regulating mitochondrial dynamics reveal potentially important aspects of the mechanism linking neuroinflammation, impaired mitochondrial function, and ASD.

FINANCIAL SUPPORT: Supported by the POWER Och!Doc Program and NSC grant 2016/23/D/NZ4/03572.

P9.1. ROLE OF A2-ADRENERGIC RECEPTORS IN VENTRAL TEGMENTAL AREA IN THE PHASIC DOPAMINE RELEASE INTO BASOLATERAL AMYGDALA IN STRESSED AND NON-STRESSED RATS

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INTRODUCTION: In the ventral tegmental area (VTA), norepinephrine (NA) promotes phasic dopamine (DA) release in the forebrain, thus regulating conditioned behaviors, such as fear conditioning. We recently demonstrated that α_2-AR blockade in the VTA reduces DA release in the mesolimbic but not mesocortical pathway. Under the influence of stress, downregulation of α_2-AR occurs in stria terminalis, therefore we hypothesize that the mechanism in VTA is similar. The net effect of α_2-AR signaling on DA release may depend on the plasticity related to factors such as acute stress.

AIM(S): The aim of this study is to demonstrate the impact of acute stress on the ability of α_2-AR signalling in stressed versus naïve, non-stressed, rats to modulate phasic DA release in the basolateral amygdala (BLA).

METHOD(S): For the study of dopaminergic signaling, fast-scan cyclic voltammetry was used in urethane anesthetized rats. The stimulation electrode, together with the guide cannula, was placed in the VTA, while the recording carbon-fiber microelectode in BLA. In the case of stressed rats, animals were subjected to fear conditioning (0.9-mA electric footshock) 24 hours before FSCV.

RESULTS: Intra-VTA administration of the α_2-AR selective antagonist RX-821002 strongly reduced phasic DA release in the BLA in naïve rats. In contrast, only modest attenuation of DA by RX-821002 was observed in the BLA of stressed animals.

CONCLUSIONS: Our results are consistent with the idea that α_2-AR signalling in various structures undergoing NAergic modulation is subject to plasticity related to stress. Here, we show that an acute stressful stimulus is sufficient to elicit this form of plasticity, potentially via α_2-AR downregulation.

P9.2. MATERNAL SEPARATION STRESS ALTERS EXCITABILITY AND STRESS INDUCED C-FOS EXPRESSION IN VENTRAL TEGMENTAL AREA DOPAMINERGIC NEURONS OF ADULT FEMALE RATS

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INTRODUCTION: Traumatic experiences in childhood and maternal separation (MS) in rodents disrupts proper development of the brain including dopaminergic (DA) mesocorticolimbic pathways originating from ventral tegmental area (VTA). Importantly, early-life stress predisposes for neuropsychiatric disorders and addiction in adulthood. Moreover, MS stress increases the number of VTA tyrosine hydroxylase (TH) immunoreactive DA neurons, raises baseline dopamine levels, and increases its release in response to acute stress in adult rats. However, neuronal mechanisms of these changes have not been fully explored.

AIM(S): The current study aimed at determining the influence of MS on VTA DA cells electrophysiology and responsiveness to acute stress at the level of c-fos expression.

METHOD(S): Female rats were submitted to MS during PND 2-14, 3 h daily. In adulthood, some of the rats were subjected to restraint stress and subsequently perfused. The VTA region was cut, stained against TH and c-fos, and double stained neurons were counted. Remaining animals were sacrificed and brain slices containing VTA were prepared for electrophysiological patch-clamp experiments. Recorded biocytin-filled cells were stained and assessed as TH+ or TH-.

RESULTS: Our data show that exposure to early life stress leads to a significant reduction of the rheobase and an increased number of action potentials generated *vs.* injected current – indices of neuronal excitability. Importantly, it was altered in both TH+ and TH- VTA neurons. MS combined with restraint stress significantly increased the number of dorsal but not ventral VTA TH+/c-fos+ cells.

CONCLUSIONS: Observed changes in excitability of VTA DA neurons may constitute a neuronal mechanism of the reported elevated dopamine release after MS. Our data indicate that MS alters reactivity of dorsal but not ventral VTA cells to acute stress, which suggests a greater raise in stress-induced DA release in structures innervated by the dorsal VTA.

FINANCIAL SUPPORT: Funding: NSC-Poland UMO--2016/21/B/NZ4/00204.

P9.3. DOES AN EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELD (50 HZ) HAVE A PERMANENT EFFECT ON STRESS-RELATED BEHAVIOUR IN RATS?

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INTRODUCTION: An electromagnetic field is a factor that people are exposed to constantly, from various sources. Thus, the interest in its influence on living organisms increases.

AIM(S): The aim of this work was to investigate the long-term effects of an extremely low frequency electromagnetic field 50 Hz (ELF-EMF) on stress-related behaviour.

METHOD(S): Adult male Wistar rats were exposed to ELF-EMF of two values of magnetic induction: 1mT and 7mT. Animals were exposed to ELF-EMF 1 or 8 hours a day for 7 days. Behavioral changes were evaluated in the open field test, which was performed in 1mT groups immediately or 5 weeks after the exposure, and in 7mT groups immediately, 5 weeks, and additionally 9 and 13 weeks after the exposure to ELF-EMF. Control rats were subjected to the same experimental procedure as the respective animals exposed to ELF-EMF except electromagnetic field exposure. We also included in the procedure the non-treated and then exposed to the open field rats (sham).

RESULTS: The behaviour of the animals exposed to 1mT did not differ significantly in comparison to control groups, regardless of the time of exposure. In 7mT groups, we found changes in stress behaviour and their intensity increased with increasing exposure time. In addition, the ELF-EMF-induced effect persisted longer in the case of the group exposed for 8 hours compared to animals exposed for 1 hour.

CONCLUSIONS: On this basis, we concluded that ELF-EMF of 7mT may act as a stress factor, and its long-lasting effects depend on the time of exposure. Furthermore, the absence of significant differences in the case of 1mT ELF-EMF may indicate the existence of certain compensatory mechanisms in the organism, which allows them to avoid a negative influence of the weak electromagnetic filed.

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P9.4. THE ROLE OF CORTISOL AND KISSPEPTIN IN SUPPRESSION OF THE GNRH/LH SECRETION IN FOLLICULAR-PHASE EWES SUBJECTED TO PROLONGED STRESS

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INTRODUCTION: A contrary relation between prolonged stress and normal reproductive efficiency has frequently been observed in domestic animals, and the action of various stressors is manifested primarily by an increase in the level of glucocorticoids in blood.

AIM(S): This study aimed to determine the central and peripheral mechanisms governing GnRH biosynthesis and LH secretion in prolonged-stressed follicular-phase sheep. They included two main experimental approaches: (1) an investigation of the effects of physical stress on GnRH and GnRH receptor (GnRHR) biosynthesis, and (2) examination of the influence of stressor on levels of mRNAs encoding kisspeptin (Kiss1) and the Kiss1 receptor (Kiss1r). Furthermore, plasma LH and cortisol concentrations were also measured.

METHOD(S): The ELISA technique was used to analyse the effects of stress on levels of post-translational products of genes encoding the GnRH ligand and GnRH receptor (GnRHR) in the preoptic area (POA), anterior (AH) and ventromedial (VMH) hypothalamus, stalk/median eminence (SME), and GnRHR in the anterior pituitary (AP). Real-time PCR was chosen for determination of the effects of stress on Kiss1 mRNA levels in the POA and the VMH the including arcuate nucleus (VMH/ARC), and on Kiss1r mRNA abundance in POA-hypothalamic structures. These analyses were supplemented by radioimmunoassay (RIA) and ELISA methods for the measurement of LH and cortisol levels in blood, respectively.

RESULTS: Stress decreased GnRH and GnRHR biosynthesis in the hypothalamus, and GnRHR in the AP. Moreover, stress lowered plasma LH concentration and levels of Kiss1 mRNA in the POA and VMH/ARC as well as Kiss1r mRNA in these structures and in the SME. An increase in plasma cortisol concentration under stress conditions was also observed.

CONCLUSIONS: This study demonstrates that stress affects GnRH/GnRHR biosynthesis and LH secretion in follicular-phase sheep, conceivably via both central and peripheral mechanisms including Kiss1 neuronal activity and action of cortisol.

P9.5. THE EFFECT OF LOW FREQUENCY ELECTROMAGNETIC FIELD (50 HZ) ON NORADRENALINE LEVELS IN THE HYPOTHALAMUS IN RATS

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INTRODUCTION: Over the last few decades, electromagnetic pollution from generated electromagnetic fields increases. Particularly important from the point of view of our health is the exposure to extremely low frequency electromagnetic fields (ELF-EMF). ELF-EMF is derived from many man-made sources, including power transmission lines or transformers. The effects induced by ELF-EMF exposure on biological systems are still unclear.

AIM(S): Therefore, the aim of this study was to determine the long-term consequences of 50 Hz ELF-EMF of 1mT and 7mT on the noradrenaline level stress parameter in the rat brain.

METHOD(S): 3-month-old male Wistar rats were divided into groups: 1) sham animals (directly taken from home cage; non-treated), or 2) animals exposed to ELF-MF (50 Hz, induction 1 mT or 7 mT). Animals were exposed to low (1 mT) or high density (7 mT) ELF-EMF for one week, for 8h/day. Control animals were subjected to the same experimental procedure as the respective animals exposed to ELF-EMF except magnetic field exposure. The level of noradrenaline in the hypothalamus was measured using HPLC immediately and 5 weeks after the exposure in the group exposed to 1mT and in the group subjected to 7mT, additionally 9 and 13 weeks after the exposure.

RESULTS: The results have shown that ELF-MF of both inductions (50 Hz) increased the level of noradrenaline in rats immediately after the exposure and the change in this hormone level was clear even 3 months later, but only in rats exposed to 7mT.

CONCLUSIONS: In conclusion, our data indicated that ELF-MF changes noradrenaline levels in the rat brain. Changes found in the present study suggest that extremely low frequency electromagnetic fields could be considered as a stress factor and can be a cause of the development of stress-related disorders.

FINANCIAL SUPPORT: Research supported by project no. 2017/25/B/NZ7/00638 National Science Centre, Poland.

P10.1. IMPLICATION OF GABA-ERGIC SYSTEM ON GNRH AND GNRHR BIOSYNTHESIS AND LH SECRETION IN THE HYPOTHALAMIC-PITUITARY UNIT OF FOLLICULAR-PHASE EWES

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INTRODUCTION: An inverse relationship between GnRH transcript level and GABA neuronal activity has been suggested; in particular, that GABA at the hypothalamic level may exert a suppressive effect on subsequent steps of GnRH biosynthesis. This inhibitory action of GABA seems to be mediated mainly *via* a GABA type A receptor ($GABA_AR$) mechanism.

AIM(S): In the present study, we analysed the effects of $GABA_AR$ activation or inhibition on the levels of post-translational products of the genes encoding the GnRH ligand and GnRH receptor (GnRHR) in the hypothalamic-pituitary unit of follicular-phase ewes using an *in vivo* infusion model.

METHOD(S): Prolonged intermittent infusions of small doses of the $GABA_AR$ agonist, muscimol, or an antagonist, bicuculline, were performed into the third cerebral ventricle of ewes. Enzyme-linked immunosorbent assay (ELISA) was used to investigate the effects of drugs on GnRH and GnRHR levels in the preoptic area (POA), anterior (AH) and ventromedial (VMH) hypothalamus, stalk/median eminence (SME), and GnRHR in the anterior pituitary gland (AP). The radioimmunoassay (RIA) method was also chosen for determining the level of LH in blood plasma.

RESULTS: The study showed that activation or blockade of $GABA_AR$ significantly decreased or increased, respectively, GnRH concentration in all analysed structures and led to analogous changes in plasma LH concentration. Similar muscimol- and bicuculline-related alterations were observed in the levels of GnRHR.

CONCLUSIONS: On the basis of these results, it is suggested that the GABAergic system in the hypothalamus of follicular-phase sheep affects GnRH and GnRHR biosynthesis either directly or via GABAergic-sensitive interneurons, leading to suppression in the secretory activity of the hypothalamic-pituitary GnRH/LH system.

P10.2. SOMATOSTATIN RECEPTORS ON PARVALBUMIN INTERNEURONS IN MOUSE SOMATOSENSORY CORTEX

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INTRODUCTION: Cortical interneurons containing somatostatin (SST-INs) are the second most numerous subtype of GABA-ergic cells in the somatosenory cortex of rodents. SST-INs inhibit excitatory cells and also other inhibitory interneurons. They are involved in disinhibitory circuits, in which SST-INs inhibit interneurons containing parvalbumin (PV-INs) which, in turn, stop inhibiting excitatory neurons. Somatostatin is present in synapses in a separate pool of vesicles and may be released together with GABA. Activation of somatostatin

receptors in the brain may cause inhibition of adenylyl cyclase, decrease of intracellular Ca^{2+} levels, hyperpolarization of cells mediated by K^+ channels, protein phosphatases activation and MAP kinases modulation. Somatostatin action on PV-INs is poorly understood.

AIM(S): To get a picture of possible sites of somatostatin action upon PV-INs, we examined the distribution of five subtypes of somatostatin receptors (SSTRs1-5) on genetically labeled PV-INs in the barrel cortex.

METHOD(S): The experiment was conducted on PV-ires-Cre driver mice lines crossed with the Ai14 line to obtain tdTomato expression following Cre-mediated recombination. Cre-dependent cell labeling was verified by immunocytochemical reaction with anti-PV antibody. A series of immunofluorescent staining using antibodies against SSTR1-5 were performed on coronal and tangential brain sections.

RESULTS: We found that SSTR1, SSTR3, and SSTR5 were present on PV-INs in all cortical layers (74% to 96% of PV neurons showed colocalization with these SSTRs). SSTR4 was found only on 36% to 62% of PV neurons, depending on the layer. Immunolabeling was found on cell bodies and dendrites. Surprisingly, we did not observe SSTR2 presence on PV-INs in any cortical layer.

CONCLUSION: Apparently, somatostatin acts on PV-INs through only four receptor subtypes, excluding SSTR2.

FINANCIAL SUPPORT: Supported by National Science Center grant OPUS 2015/17B/NZ/02016 to MK.

P10.3. INVESTIGATION OF CA^{2+} HOMEOSTASIS AND BEHAVIORAL CHANGES IN TRANSGENIC MICE OVEREXPRESSING KEY STORE-OPERATED CALCIUM ENTRY PROTEINS

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INTRODUCTION: Store-operated calcium entry (SOCE) is the major Ca^{2+} influx pathway in non-excitable cells. However, recent studies suggest its important roles in neurons. In SOCE, the depletion of Ca^{2+} from the endoplasmic reticulum (ER) causes an influx of Ca^{2+} from the extracellular space to refill the intracellular Ca^{2+} stores. STIMs are Ca^{2+} ER sensors that mediate SOCE by interacting with the ion channels in the cell membrane – ORAIs. Using transgenic mice with neuronal overexpression of STIMs and/or ORAIs, we investigate their role in neural function. Recently, we showed electrophysiological changes in hippocampi from female mice overexpressing ORAI1. Earlier studies from our group revealed increased cytoplasmic Ca^{2+} levels in cultured neurons overexpressing both ORAI1 and STIM2. Currently, we are extending these studies with the use of double transgenic STIM2/ORAI1 mice.

AIM(S): To investigate the role of SOCE proteins in neurons, and the effect of STIM2 and ORAI1 overexpression on Ca^{2+} homeostasis, synaptic functions, and behavior.

METHOD(S): We use transgenic mice that overexpress STIM and/or ORAI proteins in brain neurons. For studying Ca^{2+} homeostasis, we stain hippocampal slices with Fura-2 AM probe. To assess locomotor functions and cognitive abilities of these mice, behavioral tests are utilized. Synaptic transmission and plasticity phenomena are investigated by electrophysiological recordings from hippocampal slices.

RESULTS: We have recently observed spontaneous seizure-like events in aged female mice overexpressing ORAI1. These observations correlated with changes in the response of hippocampal slices to pro-epileptic drugs. Currently, we are focusing our analyses on the double transgenic STIM2/ORAI1 mouse line.

CONCLUSIONS: Our previous data support the view that SOCE proteins play an important role in neurons. Currently, we aim to elucidate the involvement of STIM2 and ORAI1 proteins in neural function.

FINANCIAL SUPPORT: Maestro to JK from NCN (2011/02/A/NZ3/00144).

P10.4. MOLECULAR MECHANISMS OF PROTON MODULATION IN THE GABAA RECEPTOR AS COMPARED TO LOW PH ACTIVATION SCHEME IN THE GLIC ION CHANNEL

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INTRODUCTION: The $GABA_A$ receptor ($GABA_AR$) belongs to a family of pentameric ligand gated ion channels (pLGICs). Another member of this family, bacterial GLIC, is directly activated by protons. Recent studies showed that protonation of GLIC's E35 residue starts a cascade of interactions that end at the channel pore and lead to its opening. $GABA_AR$ preserves sensitivity to protons, and ligand elicited currents can be modulated by them. The exact molecular mechanism of this action is not known.

AIM(S): This study aims to elucidate the molecular action and sensitivity of $GABA_AR$ to protons, and to answer the question of whether those mechanism are similar to proton activation in GLIC.

METHOD(S): Sequences of pLGICs were obtained from the UniProt database and aligned using T-Coffe. Additional manual refinements and alignment analysis was done in JalView. Structures of GLIC and $GABA_AR$ were obtained from RCSB PDB. Homology modeling was done using Modeller. pK_a values of $GABA_AR$ residues were assessed with PropKa 3. Structure analysis and visualization was done in VMD.

RESULTS: Using *in silico* methods, pK_a values of $GABA_AR$ residues were assessed, and we determined the positions that are homologous to proton sensitive residues of GLIC. In $GABA_AR$, no proton sensitive residues at positions homologous to GLIC E35 were detected. Instead, residues with pK_a values in proximity of physiological values was found at the cys-loop (e.g. E138 and H142 at α subunit and E150 and H156 at γ subunit), GABA binding site (βE155), upper part of ion pore (βH267 and βE270), and in its bottom part (intracellular part of the receptor, αE303 and βE313).

CONCLUSIONS: The molecular scheme of low pH activation of GLIC is not preserved in $GABA_AR$. Lack of proton sensitive residue at positions homologous to E35, and the presence of multiple possibly proton susceptible residues spread within receptor structure indicate a complex and scattered mechanism of modulation.

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P10.5. SUPERIOR COLLICULI CONTROL ACTIVITY OF THE ROSTROMEDIAL TEGMENTAL NUCLEUS IN A LATERALIZED MANNER – AN OPTOGENETIC STUDY IN THE RAT

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INTRODUCTION: Midbrain dopaminergic (DA) neuronal functioning is related to controlling the animal's orienting and movement towards salient and/or rewarding stimuli present in the environment. The firing of DA neurons is controlled by various brain regions; however, the main sensory-related innervation is brought by the ipsilateral superior colliculus (SC). Tract tracing experiments suggest that the SC also projects to the contralateral rostromedial tegmental nucleus (RMTg) – the main inhibitory input to DA neurons. Since the orientation of the animal is a manifestation of the imbalance between the left and right DA systems, it is likely that the above-described circuit might explain the lateralization of the motivational/motor behaviours toward the object located on one side of the animal.

AIM(S): The aim of this study is to describe the physiology and anatomy of the SC-RMTg circuit.

METHOD(S): Electrophysiological experiments combined with optogenetics were performed to investigate the circuit. The SC of Sprague-Dawley rats was bilaterally injected with viral vectors containing Channelrhodopsin-2 (ChR2) and yellow fluorescent protein (eYFP) genes. After ChR2 (blue light-sensitive cation channel) expression, *in vivo* electrophysiological recordings were performed. RMTg neurons were recorded using 32-channel silicon probes, while either ipsi- or contralateral SC was optogenetically stimulated with laser blue light (473 nm). After each experiment, expression of eYFP and optical fiber location in the SC, as well as, the location of the silicon probe within the RMTg, was histologically verified.

RESULTS: Obtained results revealed that stimulation of the contralateral SC was more efficient in increasing firing of the RMTg neurons, as opposed to ipsilateral SC stimulation. Additionally, ipsilateral SC stimulation was more efficient in inhibiting the firing of the RMTg neurons than contralateral SC stimulation.

CONCLUSIONS: Such brain wiring might have strong implications for the lateralisation of motivational/locomotor behaviours.

P10.6. NEUROMODULATORY CONTROL OF LONG-TERM SYNAPTIC PLASTICITY IN CA1 PYRAMIDAL NEURONS

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INTRODUCTION: Long-term synaptic plasticity (LTSP) is a complex phenomenon. Experiments utilizing different LTSP-inducing paradigms have demonstrated activation of multiple signaling pathways and uncovered differences in neuromodulatory dependence. For example, dopaminergic (D1R) activation can retroactively convert spike-timing dependent depression to potentiation. On the other hand, inhibition of beta-adrenergic (βAR) and not D1R activation can block induction of late, protein-synthesis dependent phase of LTSP (L-LTSP) evoked by rate-dependent paradigms (RTP). This is confusing because activation of both D1R and βAR increase cAMP activity and activate its targets.

AIM(S): Understand the impact of differences in temporal patterns of synaptic and neuronal activity on activation of signaling pathways and signal transduction.

METHOD(S): We used two detailed, multi-compartmental, morphologically realistic models of the CA1 neuron: 1) a conductance-based neuron model, and 2) a stochastic reaction-diffusion model of calcium-, βAR-, and D1R-activated signaling pathways underlying LTSP.

The latter model allows for simulating, monitoring, and controlling molecular concentrations in a dendritic spine and a dendritic segment.

RESULTS: Modulation of dendritic potassium ion channels (e.g., SK, Kv1.1) by protein kinase A (PKA) may explain the observed differences in neuromodulatory requirements of STDP and RTP. To predict whether paradigms eliciting spike-timing dependent plasticity will induce L-LTSP, we studied the activity of key molecules implicated in plasticity, such as calcium calmodulin-dependent protein kinase II (CaMKII) and PKA. In the spine, we studied molecular species that are involved in actin cytoskeleton remodelling, and in the dendrite – particularly those that play a role in protein synthesis.

CONCLUSIONS: These preliminary results suggest that molecular activity micro-spatial scales can predict the induction of L-LTSP.

P10.7. CREATION OF THE NEOCORTICAL LAYERS AND THEIR CONNECTIONS IN THE BRAIN OF MONODELPHIS DOMESTICA

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INTRODUCTION: In mammalian species, the neocortex contains at least six histologically-distinct layers. The main difference in the structural organization of marsupial and eutherian brains appears in interhemispheric connections. In marsupials, the biggest cerebral axonal bundle is the anterior commissure, while eutherians form an additional commissure called the corpus callosum.

AIM(S): The aim of this work was to study and compare development of the neocortex and the formation of interhemispheric connections in the opossum and the mouse.

METHOD(S): The time sequence of the formation of neocortical layers was examined using immunofluorescent staining with neuronal markers for deep layers (Tle4 and Ctip2) and upper layers (Cux1, Satb2 and Brn2). To study cortico-cortical connections, anterograde dyes, DiA and DiI were used. Generation of deeper cortical layers started at postnatal day (P)3 and continued till P8, while upper layers were generated from P9 to P19. At P9, Tle4 and Ctip2 positive neurons were observed in deep neocortical layers. Starting from P12 to P14, many cortical neurons located on the migration route between the germinal zone and upper layers were stained with Cux1, Brn2, and Satb2. At P17, Cux1 and Satb2 labeled cells reached upper layers. The pattern and proportion of Satb2 and Ctip2 positive cells in the opossum neocortex at P19 were similar to that observed in the mouse cortex at embryonic day 18. For labeling axons, DiA and DiI were injected into cortical deep layers and upper layers, respectively.

RESULTS: We found that DiA labeled axons reached the subcortical structures, while DiI labeled axons made connections between different neocortical areas.

CONCLUSIONS: The pattern of development of neocortical layers in opossums and mice is similar. However, it is shifted in time. Neurogenesis of the cerebral cortex in opossums takes place after birth while formation of the neocortex in mice occurs during the embryonic period.

FINANCIAL SUPPORT: This work was supported by grant no. 2016/22/M/NZ4/00670 from the National Science Center Poland.

P10.8. ELECTROPHYSIOLOGICAL EFFECTS OF CX3CL1 ON RAT AMYGDALA NEURONS

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INTRODUCTION: Chemokines, together with neurotransmitters and hormones, are signaling molecules that play a key role in the maintenance of the neuro-immune-endocrine system homeostasis. Accumulating evidence shows that they can modulate the activity of neurons through different mechanisms. One of their members, CX3CL1, and its cognate receptor, CX3CR1, play a crucial role in neuronal-microglia signaling.

AIM(S): As the amygdala is a relevant structure for integrating stress signaling as well as inflammatory responses from the periphery, this study aimed to elucidate the role of the CX3CL1/CX3CR1 axis on circuits within the amygdala.

METHOD(S): We used whole-cell patch-clamp and immunohistochemistry and focused on two nuclei of the amygdala: the basolateral (BLA, main input structure), and central (CeA, output structure) nuclei. Electrophysiological recordings were performed using acute brain slices (300 μm) containing the BLA and CeA. Recordings of both spontaneous inhibitory and excitatory currents (sIPSC/sEPSC), as well as, basal membrane properties of recorded cells were collected during baseline and after CX3CL1 (2nM) application. The specificity of observed effects was investigated using the same experimental protocol with additional incubation in a CX3CR1 antibody. Additionally, to specify the cell types that express CX3CR1 and CX3CL1, appropriate immunostainings were performed.

RESULTS: Our results revealed that CX3CL1 was mostly expressed within the BLA and it significantly hyperpolarized resting membrane potential of most of

the recorded principal cells (70%) and decreased their excitability; however, CX3CL1 did not alter their membrane resistance.

CONCLUSIONS: Our data show that CX3CL1 has a profound effect on synaptic activity in the rat amygdala, indicating that this protein can be an active modulator of neuronal activity in the fear-related response circuitry, which may have significant scientific and therapeutic implications.

FINANCIAL SUPPORT: Supported by National Science Centre, grant 2016/21/N/NZ4/03621.

P10.9. NMDA-INDUCED POSTERIOR HYPOTHALAMIC THETA RHYTHM RECORDED *IN VITRO*

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INTRODUCTION: Theta rhythm is one of the brain rhythms' patterns, which are evidence for neuronal synchrony. This pattern of rhythmic activity is related to sensorimotor integration, mnemonic functions, or spatial orientation and navigation. However, it is also linked to pathological conditions, for instance: Alzheimer's disease, post-traumatic stress disorder, and depression. In the last decade, we discovered that the posterior hypothalamic area (PHa) is not only a modulator of brainstem information going to the hippocampus, but also is capable of generating theta rhythm independently.

AIM(S): The aim of the present study was to determine if NMDA (N-Methyl-D-aspartic acid) is capable of eliciting well-synchronized theta activity in PHa preparations.

METHOD(S): The study was performed on 40 PHa slices prepared from 20 male Wistar rats. Each animal was anesthetized with isoflurane and decapitated. The PHa slices were dissected and transferred into the recording chamber, perfused with artificial cerebrospinal fluid, and treated with NMDA (300 μM) and D-AP5 (D-(–)-2-amino-5-phosphonopentanoic acid) (200 μM). The field recordings were performed with glass electrodes filled with 2.0 M sodium acetate.

RESULTS: Perfusions of PHa slices with 300 μM NMDA resulted in well-synchronized theta episodes which were blocked after the path application of 200 μM D-AP5.

CONCLUSIONS: The present data shows that excitation of NMDA-type glutamatergic receptors in PHa neural networks leads to the generation of local theta rhythms.

FINANCIAL SUPPORT: Supported by NCN grant no. 2017/25/B/NZ4/01476.

P10.10. GABAA β2 SUBUNIT LOOP C SHAPES BINDING AND GATING PROPERTIES OF RECEPTOR ACTIVATION

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INTRODUCTION: $GABA_A$ receptors ($GABA_ARs$) are pentameric ligand-gated ion channels that are crucial in fast inhibitory transmission in adult CNS. The activation process of $GABA_AR$ couples agonist binding to the binding site in the extracellular domain, with an opening of the channel gate in the far-distant transmembrane domain. $GABA_AR$ gating is a complex process that includes preactivation (flipping), which is a step of bound receptor that remains closed and precedes opening. Our recent study shows that flipping is modulated by benzodiazepines. A key element in $GABA_ARs$ activation is loop C capping, an inward movement of the β2 subunit loop C upon ligand binding; however, the exact role of loop C in relation to $GABA_AR$ binding and gating remains elusive.

AIM(S): This study aims to explain how a mutation of the β2F200 residue in loop C affects functioning of the $GABA_AR$ in reference to receptor binding and gating, including modulation of preactivation by flurazepam (benzodiazepine).

METHOD(S): β2F200 mutated receptors (Tyr, Ile, Cys) were expressed in HEK293 cells. Patch clamp was used to record macroscopic currents elicited by saturating [GABA] (combined with ultrafast perfusion system) and single channel currents. Kinetic analysis was preformed and followed by kinetic modeling.

RESULTS: β2F200 mutants exhibited a shift in dose-response relationship. The mutation significantly slowed down current onset and desensitization, but deactivation was accelerated. Flurazepam potentiated currents evoked by saturating [GABA] in contrast to WT receptors. Single-channel analysis showed a significant change in all shut time distributions components and shortening of open time distributions.

CONCLUSIONS: Kinetic modeling of macroscopic and single channel currents confirmed alteration in all considered gating properties. *In silico* ligand docking indicated a drop in the binding affinity for each mutant. $GABA_AR$ loop C plays a critical role in receptor binding and gating.

FINANCIAL SUPPORT: NCN grant 2015/18/A/NZ1/00395.

P10.11. ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE RAT NUCLEUS INCERTUS NEURONS IN RELATION TO HIPPOCAMPAL THETA OSCILLATIONS

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INTRODUCTION: The nucleus incertus (NI) is a bilateral structure located adjacent to the midline of the brainstem, below the fourth ventricle. NI is formed of GABAergic neurons strongly innervating theta pacemaker regions of the brain. Previously, it has been shown that activation of NI induces hippocampal theta rhythms, whereas inactivation impairs it. The NI itself is a part of a network working at theta frequency. However, the electrophysiological characteristics of NI neurons and their involvement in the mechanisms of theta rhythm generation are unclear.

AIM(S): Our goal was to determine the classification of NI neurons based on their electrophysiological properties in relation to hippocampal theta oscillations.

METHOD(S): The experiments were conducted on Sprague-Dawley rats under urethane anaesthesia that induces cyclic alternations of brain states (dominance of theta oscillations or slow waves). Neuronal activity was recorded extracellularly using a 32-channel recording system in combination with acute microelectrode arrays. At the same time, theta rhythm and slow wave activity were recorded from the stratum lacunosum-moleculare layer of the hippocampal CA1 field.

RESULTS: We have shown that the level and pattern of NI neuronal firing is brain state dependent. Two main groups of NI neurons could be distinguished: theta-phase locked cells (46%, 66/145), and theta-phase independent cells (54%, 79/145). A majority of theta-phase locked NI neurons are characterized by rhythmic bursting with a strong preference to fire action potentials at the rising phase of hippocampal theta oscillation (theta bursting neurons; 68%, 45/66).

CONCLUSIONS: We have discovered that NI neuronal activity patterns are more complex than has been previously described. Almost a third of all recorded NI neurons exhibit a theta-bursting pattern of firing, suggesting that the NI not only modulates theta oscillations but is itself an oscillator.

P10.12. STIM2 ROLE IN RESPONSE TO OXIDATIVE STRESS

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INTRODUCTION: STIM1 and STIM2 proteins are calcium sensors residing in the ER. They are involved in the regulation of SOCE (Store-Operated Calcium Entry) and take part in Ca^{2+} homeostasis and signaling. They participate in the regulation of numerous processes such as genes expression, neurotransmission, and neuronal morphology. STIM1 is also sensitive to redox state and may regulate functions of mitochondria. STIM2 is highly expressed in the neuronal tissue and was shown to be involved in processes leading to neurodegeneration. Oxidative stress and abnormal activity of mitochondria are frequently proposed to be a part of the mechanism that leads to the neurodegeneration.

AIM(S): We aim to check the role of Stim2b zebrafish isoform in the activity of mitochondria, and its sensitivity to redox state.

METHOD(S): Wild-type and *stim2b*$^{-/-}$ zebrafish are being used in the experiments and oxidative stress is induced by 2 mM H_2O_2 treatment of 5dpf larvae. These fish lines express, specifically in neurons, a genetically encoded Ca^{2+} probe – GCaMP5G – that allows us to track changes in Ca^{2+} levels in response to oxidative stress. We use state-of-the-art techniques of *in vivo* Ca^{2+} imaging using lightsheet microscopy and qPCR to analyze gene expression.

RESULTS: We found that expression of genes (like catalase), that are known to be involved in response to the oxidative stress, was different in *stim2b*$^{-/-}$ fish as compared to wild-type in untreated larvae. mRNA level of catalase remains elevated in mutants also after oxidative stress induction.

CONCLUSIONS: These data indicate that Stim2b might be involved in the brain response to oxidative stress. However, calcium imaging data, which are now being collected *in vivo*, are needed to confirm this hypothesis.

P10.13. SCREEN FOR KINESINS CRITICAL IN MTOR-DEPENDENT NEURONAL DEVELOPMENT

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INTRODUCTION: The mammalian target of rapamycin (mTOR) has an important role in the maturation of developing neuronal cells. It can be seen in the condition of mTOR hyper-activation, which leads to premature differentiation, uncontrolled overgrowth of the dendritic tree, and elevated cell soma sizes in *in vitro* cultures. In patients, this phenotype leads to diseases like tuberous sclerosis complex, which results in brain tumors, epilepsy, mental retardation, and autism spectrum disorder. In highly polarized cells like neurons, growth and proper development are highly dependent on proper cellular transport, as cargo must be delivered to distant parts of cells especially dendrites and axon.

Dynein-dynactin complex and kinesins are motor proteins that play a crucial role in this process.

AIM(S): While recent publications show a dependence between dynein and mTOR stimulated growth, not much is known about the interplay between kinesins and mTOR in this process.

METHOD(S): A primary screen for kinesins critically involved in mTOR-dependent neuron outgrowth was performed, with the use of a shRNA library targeting 38 different kinesin heavy chain genes (*KIF*s), while simultaneously stimulating mTOR by PI3-kinase pathway. Data was collected by transfecting primary rat neuronal cultures *in vitro*, and measuring soma size and dendritic tree arborization on images taken five days after the transfection. Based on phenotyping of cells after silencing, 10 *KIF* genes were selected for further investigation.

RESULTS: While in almost all cases, silencing of *KIF* genes resulted in altered neuron phenotype, this may be due to the importance of kinesins in proper cytoskeleton formation. In some of the genes, however, the phenotype was more similar to wild-type neurons in spite of stimulating the PI3K pathway.

CONCLUSIONS: Further experiments with neuronal stem cells differentiation, neuro-progenitor migration, and biochemical analysis will be performed to investigate the relation of the selected genes to the mTOR pathway.

P11.1. ACUTE NORMOBARIC HYPOXIA LOWERS EXECUTIVE FUNCTIONS DESPITE AN INCREASE IN BDNF LEVELS

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INTRODUCTION: Although hypoxia has the potential to decrease SpO_2 while ascending to high altitudes, which can cause impairments in cognitive functioning, the effects of acute hypoxia on high-order brain functions like executive processing remain unclear.

AIM(S): The purpose of this study was to investigate the acute response to normobaric hypoxia and its effect on executive function and changes in BDNF (Brain Derived Neurotrophic Factor), as a protein that modulates brain health and cognition.

METHOD(S): Thirty-two healthy subjects participated in a blind study where they performed two sessions of single 30 min breathing bouts under two conditions (normoxia (NOR) and normobaric hypoxia (NH)), on different days. On the first session, they breathed ambient air and on the second session, participants breathed hypoxic air (fraction of inspired oxygen (FIO_2) = 0.135), which corresponded to an altitude of 3500 m. Before and after both sessions, participants performed the color-word Stroop task and level of SpO_2 was monitored.

RESULTS: There was no significant difference in Stroop interference in the "reading" part of the test in either conditions compared to baseline, but there was a significant increase in the "naming" part of the Stroop interference test in NH conditions ($p=0.0056$), which corresponded with a significant decrease in SpO_2 ($p<0.0001$). There was also a significant increase ($p<0.0001$) in BDNF levels after NH conditions as compared to the baseline, what could not be seen in NOR.

CONCLUSIONS: These results suggest that acute hypoxia impaired neural activity in motor executive and inhibitory processing and delayed cognitive processing for motor execution. Hypoxia has the potential to impair cognition, but the effects of acute hypoxia on cognitive function remain debatable. We investigated the effects of acute exposure to moderate hypoxic conditions, and observed decreased executive function and this negative effect was associated with decreased SpO_2, irrespective of a BDNF rise.

P11.2. IMPACT OF KETOGENIC DIET ON ENTEROCOCCUS FAECALIS IN THE GUT FLORA OF LONG EVANS AND WISTAR RAT STRAINS

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INTRODUCTION: The ketogenic diet (KD) is used to manage drug-resistant epilepsy in children. The KD directs the metabolism towards fat consumption as an alternative source of caloric demand, thus a state similar to starvation is created. The gut-brain axis is associated with biochemical signaling between the large intestine and the nervous system via gut microbiota. It seems that, for an efficient functioning of the brain, a well-developed gut-brain axis is required. Growing evidence supports the participation of gut microbiota in the regulation of cognitive functions, mood, anxiety, and pain. The probability of the KD affecting the composition of gut microbiota has been shown. One of the important aspects is the right choice of animal model for research. Animal models are not equal in metabolic, biochemical, physiological, and behavioral conditions. It is important to know the microbiota diversity in animal models.

AIM(S): The aim of this study was a quantitative and comparative analysis of *Enterococcus faecalis* bacteria in the faeces of Wistar and Long Evans rats fed with KD of two different compositions.

METHOD(S): Ten male Wistar rats and ten male Long Evans rats were divided into two groups. Animals were fed the KD based on animal or plant fat for 28 days. Subsequently, real-time PCR was performed to assess the number of *Enterococcus faecalis*. Body mass and β-hydroxybutyrate levels were measured as well.

RESULTS: It has been observed that, after 28 days, the number of *Enterococcus faecalis* in faeces of rats fed with the KD was increased. Furthermore, results showed an increase in body mass and β-hydroxybutyrate levels in both Wistar and Long Evans rats.

CONCLUSIONS: In conclusion, analysis of changes in the number of bacteria *Enterococcus faecalis* has shown that the KD may alter gut microbiota composition in Long Evans and Wistar rats fed with the KD.

P11.3. THE INFLUENCE OF KETOGENIC DIETS ON THE AMOUNT OF ENTEROCOCCUS FAECALIS AND ESCHERICHIA COLI IN FAECES OF THE 129SV MICE

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INTRODUCTION: The ketogenic diet (KD) is high fat and low carbohydrate diet type. Ketogenic metabolism is characterized by moderate hypoglycemia. It also leads to the use of ketone bodies as an alternative source of energy for the organism. In the last decade, the influence of various factors on the gut microbiota has been noticed. The gut microbiota includes bacteria as well as fungi, archaea, viruses, and protozoa. Gut microbiota has been shown to participate in the regulation of processes in the central nervous system via the gut-brain axis. Differences in the composition of gut microbiota can affect the proper functioning of the central nervous system. It seems that KD can affect the gut microbiota composition.

AIM(S): The aim of the study was to evaluate the influence of two types of KD high in animal fat on the number of *Enerococcus faecalis* and *Escherichia coli* in faeces of 129SV mice.

METHOD(S): Ten male 129SV mice were divided into two groups and fed for 28 days with a KD high in animal fat with different ratios (4: 1 and 6: 1). The assessment of the number of bacteria was performed on 0, 14, and 28 days of feeding with the KD by real time-QPCR. Additionally, body mass and β-hydroxybutyrate levels were measured.

RESULTS: An increase of the amount of *Enterococcus faecalis* and *Escherichia coli* after 14 and 28 days in the faeces of 129SV mice fed with both KDs was observed. The results showed a decrease in the body mass of mice fed with KD with ratio of 4: 1. Changes in body mass of mice fed with KD with a ratio of 6: 1 has not been observed. The increase of β-hydroxybutyrate levels in blood of mice fed with both KDs has been noticed.

CONCLUSIONS: In conclusion, results of evaluation of the number of *Enterococcus faecalis* and *Escherichia coli* may suggest that a KD high in animal fat affects the gut microbiota composition in 129SV mice.

P11.4. THE ABILITY OF BDNF TO DECREASE CASPASE-3 LEVEL DEPENDS ON BODY TEMPERATURE UNDER ANOXIC CONDITIONS

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INTRODUCTION: The neuronal cell death associated with perinatal anoxia plays a significant role in neonatal morbidity and neurodevelopmental disability. In response to mild stress, a number of compensatory mechanisms are activated, which allows the cells to survive. Beside decreased body temperature, brain-derived neurotrophic factor (BDNF) is also considered to be beneficial to neuronal survival.

AIM(S): Therefore, the aim of this study was to determine whether body temperature under anoxic condition affects the ability of BDNF (proBDNF and mBDNF) to decrease caspase-3 levels in the developing brain.

METHOD(S): 2-day-old Wistar rats were divided into 3 temperature groups: i) normothermic -33°C (typical body temperature of newborn rats), ii) hyperthermic – 37°C (typical body temperature of adult rat), and iii) extremely hyperthermic – 39°C (typical body temperature of febrile adult rats). The temperature was controlled starting 15 minutes before, and the measurement was continued during 10 minutes of anoxia (pure nitrogen atmosphere), as well as, for 2 hours postanoxia. Levels of BDNF and caspase-3 were determined *post mortem*, 2 and 72 hours after anoxia using Western blot and ELISA analysis.

RESULTS: Body temperature affected the levels of endogenous BDNF, its precursor form (proBDNF), and caspase-3. In anoxic animals, the levels of proBDNF and caspase-3 increased with increasing neonatal body temperature. In contrast, a significant negative correlation between the total BDNF to proBDNF ratio, and caspase-3 concentrations was observed.

CONCLUSIONS: The results suggest that decreased body temperature can not only up-regulate BDNF levels, but also may affect the other functions of this neuropeptide.

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P11.5. EFFECT OF AEROBIC AND RESISTANCE INTERVAL EXERCISES ON PERIPHERAL CONCENTRATION OF NEUROPROTECTIVE PROTEINS AND HUMAN COGNITIVE ABILITIES

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INTRODUCTION: A growing number of human studies suggest that regular physical activity can improve not only physical but also mental health. Most interventions are based on continuous-aerobic-mild to moderate intensity exercise, which requires people to make a sufficient time commitment and are often indicated as less enjoyable and boring, which is a known limiting factor. Given the markedly lower training volume, interval training is a time-efficient strategy that induces rapid muscle and cardio-respiratory adaptations. Therefore, the interval exercise protocol was successfully applied in many health-oriented programs focusing on the prevention of metabolic and cardio-respiratory diseases. However, studies evaluating the effects of interval exercise on brain functions are limited.

AIM(S): The aim of this study was to investigate whether aerobic and resistance interval exercises affect the peripheral concentration of selected neuroprotective proteins (BDNF, IGF-1, VEGF), as well as, modulate human cognition.

METHOD(S): The study involved Gdansk University of Physical Education and Sport students. The main experiment consisted of three trial sessions, control (CON), aerobic high-intensity interval exercise (AHIE), and resistance high-intensity interval exercise (RHIE), separated by at least one week. Before and after interval exercises, a growth factor (BDNF, IGF-1, VEGF) assay was applied using the ELISA method. An auditory verbal paired-associate learning task was used to assess memory.

RESULTS: Both interval exercise protocols modulate peripheral concentration of selected neuroprotective proteins. Obtained results of cognitive functions indicated that acute interval exercise significantly improved memory in young adults.

CONCLUSIONS: The results indicate that the proposed interval exercise can induce beneficial changes in human cognition through an increase in peripheral neuroprotective protein concentrations.

P11.6. ANXIOLYTIC EFFECT OF PHYSICAL ACTIVITY AND CEREBRAL ACCUMULATION OF SATURATED FATTY ACIDS

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INTRODUCTION: The broad spectrum of the positive effects of physical activity on brain functioning is well acknowledged. Among others, it induces an improvement in mood, and a part of the rodent studies support this thesis by showing anxiolytic effects of exercise. However, the mechanism of this behavioral modification is not clear. Changes in brain metabolism may contribute to the generation of complex brain disorder phenotypes; thus, metabolomics have proven to be useful tools in studies on the central nervous system.

AIM(S): The discrimination of anxiolytic level and metabolomics changes in the brain were evaluated in this study.

METHOD(S): Voluntary running mice were subjected to a battery of behavioral tests (Open Field, Elevated Plus Maze, Dark/Light Box) commonly used to measure anxiety levels. Simultaneously, GC/MS analysis of hippocampal and cortical samples was performed for metabolome profiling of the running mice.

RESULTS: The exercised animals showed anxiolytic behavior. Voluntary running caused an accumulation of saturated fatty acids, such as myrisitc, palmitic, heptadecanoic, and stearic acids, in the hippocampus and cortex of running mice.

CONCLUSIONS: A striking observation in the present study is that a profile of saturated fatty acids that accumulates in the hippocampi and cortex of the running mice is consistent with the mixture of fatty acids that was identified as causing anxiolytic-like effects when administered to rodents.

P11.7. KETOGENIC DIETS BASED ON FAT OF EITHER ANIMAL OR PLANT ORIGIN HAVE DIFFERENT EFFECTS ON THE ABUNDANCE OF AUTOPHAGIC VESICLES IN MOUSE BRAIN

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INTRODUCTION: Autophagy is a cellular recycling mechanism essential for maintenance of cell homeostasis and viability, especially during stress conditions; hence, autophagy is involved in a number of physiological and

pathological processes. Autophagy is thought to be involved in anti-aging and neuroprotective effects of caloric restriction, Sirtuin 1 activation, inhibition of insulin/insulin-like growth factor signaling, and administration of rapamycin, resveratrol, and metformin. The ketogenic diet mimics the biochemical actions of fasting and exerts many physiological and cellular responses similar to those evoked by intermittent energy restriction. Despite this, the relationship between nutritional ketosis and autophagy has been a largely unexplored field.

AIM(S): The aim of this study was to verify the hypothesis that ketogenic diets affect the process of autophagosome formation in the hippocampus and/or cerebral cortex.

METHOD(S): 9-week-old male mice were fed with one of two differently composed ketogenic chows – based on the fat of either animal or plant origin (KA, KP respectively) or with standard rodent chow (SD) – for 6 subsequent weeks. Western blotting, (LC3, p62), QRT-PCR (LC3A, LC3B, p62), and confocal microscopy (LC3 puncta) were employed to monitor autophagy in hippocampal and cerebrocortical samples.

RESULTS: Western blot results revealed increased levels of LC3 II protein – a marker of autophagosomes – in the hippocampus and frontal cortex of mice treated with the ketogenic diet. This observation was confirmed by the evaluation of a number of LC3 puncta with immunofluorescence microscopy. The size of this effect was dependent on the composition of the diet.

CONCLUSIONS: This study reports, for the first time, an upregulation of autophagosome synthesis in the brain of animals fed with the ketogenic diet. Our results make a significant contribution to the understanding of the mechanisms of ketogenic diet action.

FINANCIAL SUPPORT: This research is supported by the National Science Center grant no. 2017/01/X/NZ3/00984.

P11.8. EFFECTS OF DIET-INDUCED OBESITY AND DIABETES TYPE 2 ON NEUROPEPTIDE Y-IMMUNOREACTIVE NEURONS IN THE HYPOTHALAMUS OF MALE AND FEMALE RATS

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INTRODUCTION: Obesity is a global issue and a major metabolic disorder, leading to the development of serious diseases such as diabetes type 2 (DM2). Importantly, DM2 accounts for over 90 % of the diabetic cases in human. There are also sex-specific differences in the development of obesity and DM2. The arcuate nucleus of the hypothalamus (ARC) is believed to be central to integration of peripheral and central anabolic and catabolic inputs and the maintenance of energy balance. Neuropeptide Y (NPY) neurons plays a role in the regulation of food intake and modulation of energy expenditure. In rat brain, NPY neurons are located in the ARC and send projections to numerous other hypothalamic regions. However, there is no data concerning the comparison of the role of diet-induced obesity and DM2 on these neurons in male and female rats.

AIM(S): The aim of this project was to study the effects of obesity and DM2 on NPY-immunoreactivity in the ARC of the hypothalamus of male and female rats.

METHOD(S): To induce obesity female and male Wistar rats were fed with a high fat diet (HFD). In order to mimic the DM2 condition, a subset of HFD animals were injected with streptozotocin (a toxin that destroys pancreatic cells). The control group received lab chow diet. Animals were sacrificed, perfused with paraformaldehyde, and blood and brains were collected for further analysis. Blood was used to assess the metabolic and hormonal status of the animals and brains were processed for immunohistochemistry.

RESULTS: Analysis of metabolic and hormonal status confirmed induction of obesity and DM2 in animals. Preliminary data (n=3) indicate an increase in NPY-immunostaining in the ARC of obese male and female rats compared to controls. Currently the remaining data are being analyzed.

CONCLUSIONS: The preliminary data indicate that HFD-induced obesity may alter functions of NPY neurons in the ARC of male and female rats.

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P11.9. ANXIETY-LIKE BEHAVIOR OF LABORATORY ANIMALS ON A KETOGENIC DIET

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INTRODUCTION: In the recent years, more attention is attributed to the impact of diet on central nervous system function. An increasing number of diseases, including neurological disorders, results from inadequate dietary habits. Diet, however, can affect the function of brain and mental processes in a negative as well as a beneficial way. The diet with well-documented neuroprotective effects is a high-fat and low-carbohydrate ketogenic diet (KD).

METHOD(S): We investigated two type of KD, one of them based on animal (KDA), while the other on vegetable fats (KDB). Both diets were applied to two groups of laboratory animals: mice (129S2/SvPasCrl) and rats (Long-Evans Rat, Crl: LE) for 6 weeks.

CONCLUSIONS: Interestingly, preliminary data indicate a unique anxiogenic action of the KD but only in mice.

P11.10. EFFECTS OF OVARIECTOMY AND SEX HORMONE REPLACEMENT ON THE NUMBER OF NEUROKININ B-IMMUNOREACTIVE NEURONS IN THE ARCUATE NUCLEUS OF THE HYPOTHALAMUS OF OBESE AND DIABETIC FEMALE RATS

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INTRODUCTION: The reproductive capacity of mammals is governed by the hypothalamus-pituitary-gonadal (HPG) axis, with gonadotropin-releasing hormone (GnRH) localized on top of it. Neuronal population expressing kisspeptin, neurokinin B (NKB), and dynorphin (KNDy neurons), present in the arcuate nucleus (ARC) of the hypothalamus, are important for regulating GnRH secretion. Both obesity and type 2 diabetes (DM2) are major risk factors for reproductive alterations (e.g., decreased fertility). Because ARC is a site where cross-talk between metabolism and reproduction occurs, those states may influence KNDy neurons. However, data on the role of metabolic imbalance, gonadectomy, and sex steroid replacement in the regulation of NKB expression are limited, especially in females.

AIM(S): The aim of this study was to assess the effects of metabolic disruption (high-fat diet-induced and DM2), ovariectomy, and sex hormone replacement on the number of NKB-immunoreactive (-ir) neurons in the ARC of female rats.

METHOD(S): Female rats received a control (C) or high-fat diet (HFD) for 13 weeks. Streptozotocin injections were performed to induce DM2 in half of the animals from the HFD group. The following groups were obtained: C, HFD, and DM2. Then, animals were divided into three subgroups: ovariectomy (OVX), ovariectomy and estradiol replacement (OVX+E_2), and ovariectomy together with estradiol and progesterone replacement (OVX+E_2+P_4). Metabolic profile was assessed and immunohistochemistry for NKB was performed.

RESULTS: There was an effect of operation ($p<0.01$). In C and DM2, there was a higher number of NKB-ir cells in OVX+E_2+P_4 *vs.* OVX. Additionally, in the DM2 group, a higher number of NKB-ir was seen in OVX+E_2 *vs.* OVX.

CONCLUSIONS: HFD does not change the response of NKB-ir neurons to OVX and hormonal replacement. However, in DM2 females, NKB-ir neurons are more sensitive to the OVX+E_2 condition.

FINANCIAL SUPPORT: Supported by NCN grant 2015/17/B/NZ4/02021.

P11.11. THE INFLUENCE OF VARIOUS COMPOSITIONS OF THE KETOGENIC DIET ON THE BEHAVIOR OF SELECTED MOUSE STRAINS

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INTRODUCTION: High fat diets are characterized by a high fat content with an adequate proportion of proteins and carbohydrates. For a long time, the ketogenic diet has been used in the treatment of epilepsy; however, its neuroprotective properties indicate its putative and broader application in the treatment of neurodegenerative diseases and neurodevelopmental disorders.

AIM(S): The present study is focused on the selection of a proper animal model in the preclinical studies on ketogenic therapeutic potential.

METHOD(S): The study involved 160 male mice from 129/SV and C57 BL/6 strains at the age of 9 weeks. The animals were divided into 4 groups. The control groups have been fed with a standard diet and the experimental groups were fed with three different types of a high fat diet: (A) a diet imitating a classic ketogenic diet composed mainly of saturated fats of animal origin, where the ratio of fat to carbohydrates and proteins is 4:1, (B) a diet imitating a modified ketogenic diet with a high content of unsaturated fat of vegetable origin, where the ratio of fat to carbohydrates and proteins is 4:1, or (C) a diet imitating ketogenic diet, containing fats of animal origin where the ratio of fats to carbohydrates and proteins is 6:1. On the 100th day of the experiment, the animals of all groups were subjected to motor and behavioral tests, including: an open field test, elevated plus maze, test on a raised treadmill, and a Grip Test. After testing, the animals were euthanized to collect material for further analysis.

CONCLUSIONS: The collected samples will be used to determine the level of expression of selected genes and the level of neurogenesis in the hippocampal dentate gyrus.

AUTHOR INDEX