Course 3

Basis of rodent behavioral research

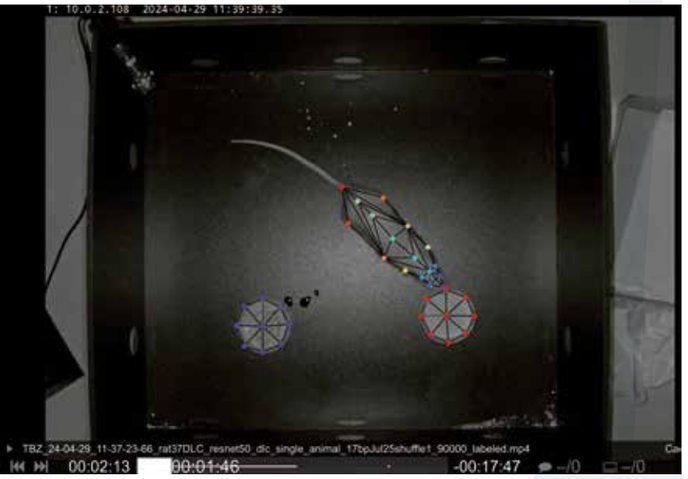
An introduction to rodent behavioral studies' design, conduct and interpretation.

PANERIS

PAN-EUROPEAN NETWORK FOR NEUROSCIENCE RESEARCH INFRASTRUCTURE AND STRENGTHENING OF SUPPORT CAPACITIES (PANERIS)

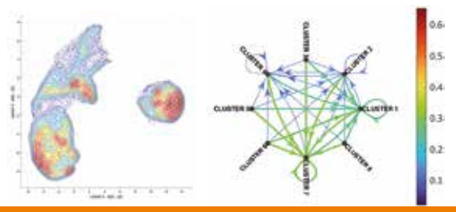
Workshop topics:

The primary goal of the workshop is to familiarize participants with key aspects of each stage in rodent behavioral research. The course covers theoretical foundations, study design and preparation, experiment execution, data analysis, and result interpretation. Participants will begin with an overview of rodent behavior principles and a range of available assays. For hands-on practice, they will conduct a selected behavioral assay, working through each phase of experiment preparation and execution. Additionally, participants will be introduced to innovative behavioral approaches, including rodent ultrasonic communication and the use of deep-learning algorithms in behavioral analysis.



Workshop techniques and equipment:

- **Audio tools**
  ultrasounds recording (Avisoft®) and analysis (DeepSqueak)
- **Video tools**
  DeepLabCut for the assessment of rats' behavior



Tutors:

Dr. Agnieszka Potasiewicz – Agnieszka is a behavioral neuro scientist in the Department of Behavioral Neurosciences and Drug Development, where she explores social and cognitive phenomena in rodents to identify new treatment alternatives. She earned her PhD studying how alpha7 nicotinic acetylcholine receptor ligands might improve cognitive symptoms in animal models of schizophrenia. Her current research focuses on rodent ultrasonic communication in different neurodevelopmental conditions corresponding to autism and schizophrenia.

MSc. Ewelina Cyrano – Ewelina is a behavioral neuroscientist and IT specialist in the Department of Behavioral Neurosciences and Drug Development. She graduated from the Faculty of Biology at Jagiellonian University. Ewelina excel in building and applying deep-learning algorithms of DeepLabCut and SimBA models to evaluate rodent behavior. Now, her research interests focus on the analysis of small movement patterns.

Dr. Natalia Malikowska-Racia – Natalia is a behavioral neuroscientist in the Department of Behavioral Neurosciences and Drug Development. With over 10 years of experience in the field, she has gained expertise at institutions such as Philipps-Universität Marburg and the University of Catania. She graduated from the Jagiellonian University Medical College, where she completed her PhD on traumatic stress and classical conditioning in mouse models. Her current research focuses on rat operant learning models of decision-making and motivation.

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PANERIS Winter School

Addiction: **underlying mechanisms and search for novel therapies**

Maj Institute of Pharmacology Polish Academy of Sciences

3rd – 7th March 2025, Kraków, Poland

IDIBAPS Institut d'Investigacions Biomèdiques August Pi i Sunyer

Instytut Farmakologii im. Jerzego Maja Polskiej Akademii Nauk

Instituto de Medicina Molecular | João Lobo Antunes

Vilniaus universitetas

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Course 1

High-resolution imaging of stress-induced synapsis plasticity

An introduction to confocal microscopy.

Workshop topics:

During the workshop, participants will be introduced to the applicability of the high-resolution imaging technique - confocal microscopy to study the plasticity of synapses induced by chronic stress. The workshop will include a theoretical introduction to microscopic imaging and to the method of induction of a chronic stress model in primary cultures of cortical and hippocampal neurons. Participants will then have the opportunity to perform experiments in the laboratory: from treating primary cultures with stress-inducing agents, staining cells to capturing high-resolution images on a confocal microscope. Finally, as part of the workshop, participants will be able to analyze the acquired images in dedicated open-source software for analyzing this type of microscopic data.

Workshop techniques and equipment:

Confocal Microscope TCS SP8X (Leica)

Confocal Microscope Leica TCS SP8 X: an inverted microscope equipped with a white light laser and hybrid HyD and PMT detectors. It allows excitation of any fluorophore in the 405 nm - 600 nm range, with spectral detection and dual imaging options (wide-field and confocal). An environmental chamber maintains optimal conditions for long-term live cell imaging, enabling FRAP, FLIP and FRET experiments.

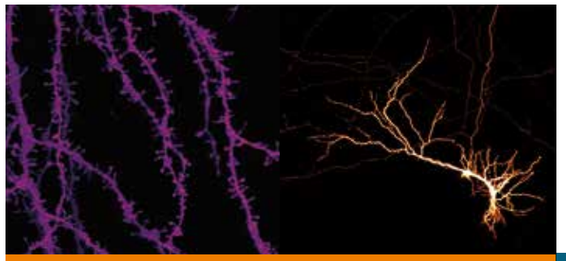
Tutors:

Dr Julita Wesołowska – Head of the Laboratory of Microscopic Imaging, which she has been in charge of since 2018. She is also affiliated with the Faculty of Biochemistry, Biophysics and Biotechnology at the Jagiellonian University, where she teaches and pursues research interests. She is interested in DNA double-strand break repair processes, which she studies using confocal microscopy. She defended her PhD focusing on the characterisation of 53BP1 protein repair foci formed in response to CRISPR/Cas9 or visible light-induced DNA double-strand breaks. During her doctoral studies, she held internships at research institutions (Institute of Molecular Biology in Mainz) and in industry (Selvita S.A. in Kraków).

MSc. Michał Wilczkowski – PhD student at the Maj Institute of Pharmacology PAS, with a keen interest in the molecular mechanisms underlying dendritic spine plasticity. His current research delves into the role of focal adhesion kinase in mediating synaptic plasticity induced by corticosterone.

For more information, please visit:

https: //paneris.eu/



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Course 2

Application of mass spectrometry in proteomics

Data-independent Acquisition 4D Proteomics – the knowledge beyond protein identification.

Workshop topics:

The aim of the course is to familiarize participants with methods of preparation of biological samples for proteomic analyses, including protein digestion in polyacrylamide gel, in solution, FASP and SP3 techniques. During the classes, DDA and DIA measurement techniques will be discussed and participants will be familiarized with the available software for the analysis of such data and the biostatistical processing of the obtained results. The classes will include individual bottom-up measurements using a MALDI-TOF/TOF spectrometer and demonstration classes in 4D proteomics using the timsTOF Pro spectrometer.

Workshop techniques and equipment:

TimsTOF Pro 2 (Bruker) – a high resolution time-of-flight mass spectrometer with TIMS analyser

The timsTOF Pro 2 mass spectrometer is a state-of-the-art, high-resolution instrument unique in Poland. It combines a highly sensitive ESI ion source with a TIMS (Trapped Ion Mobility Spectrometry) analyzer, improving proteomic studies' structural data and quality. It excels in shotgun proteomics, especially when combined with capillary liquid chromatography using PASEF technology. This versatile setup supports applications beyond proteomics, including drug analysis, endogenous compound studies, and quantitative analysis.

UltrafleXtreme (Bruker) – a high-resolution time-of-flight mass spectrometer with MALDI ion source

This instrument offers top performance in proteomics, biomarker research, and MALDI molecular imaging (MALDI-IMS). It enables accurate identification and quantification of proteins using LC-MALDI with isotope labeling methods such as iTRAQ and ICAT, allowing the simultaneous analysis of thousands of proteins. It also measures intact protein masses and their complexes and supports tissue imaging using MALDI to map the spatial distribution of biomolecules such as lipids, proteins, and small molecules.

Tutors:

Przemyslaw Mielczarek, PhD – Founder of the Proteomics and Mass Spectrometry Laboratory at the Jerzy Maj Institute of Pharmacology in Krakow and Lecturer at the Department of Analytical Chemistry and Biochemistry at AGH University. He specialises in DIA-based proteomics and molecular tissue imaging using MALDI-TOF/TOF, and his research focuses on the application of mass spectrometry in the study of biomolecules related to drug addiction. He also completed an internship at the Ludwig Institute for Cancer Research in Sweden. He worked with Prof. Ulf Hellman, an expert in MALDI mass spectrometry.

Kinga Szafran-Pilch, PhD – Completed her master's degree in biology with a specialization in cell biology at the Jagiellonian University and subsequently defended her PhD in medical sciences, specializing in neuropsychopharmacology, at the Maj Institute of Pharmacology PAS. Her research interests focus on studying the role of G protein-coupled receptors dimerization and GPCR-mediated signaling pathways.

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