MAGNIFICENT SEVEN 3

IŁAWA 15 - 17 AUGUST 2021

15 AUGUST

15:30 – 16:00 Coffee and snacks

16:00 – 18:00 Session I - Stress & Behavioral Research

Molecular Fingerprint of stress resilience
Ewa Bączyńska
Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland

Stress, Microbiota-Gut-Brain-Axis and Nutrition
Daria Guseva
Institute of Nutritional Medicine, University of Hohenheim, Germany

Combination of Dasatinib and Quercetin improves cognitive abilities in aged Wistar rats
Gregory Petrazzo
Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland

19:00 Dinner

16 AUGUST

11:00 – 13:00 Session II - Advanced Research Methods

Visualizing of Topologically Associated Domains using Three-Dimensional Electron Microscopy In Situ Hybridization
Błażej Ruszczycki
Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland

Generation of human neurons from induced pluripotent stem cells for potential screening of drug candidate for neuropsychiatric diseases
Elise Tse
Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland

Efficacy of selected inhibitors of protein S-palmitoylation in synaptic plasticity
Agata Pytyś
Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland

SNO-TRAP synthesis and possible applications
Marta Czarnota-Bojarska
Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland

13:00 Lunch
19:00 Dinner

Organizers:
Monika Bijata
Jakub Włodarczyk
Ewa Bączyńska
Anna Bartkowiak-Kaczmarek

Ewa Bączyńska (Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland)

Molecular Fingerprint of stress resilience

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Stress resilience is the ability to be exposed to a stressful experience and cope with it. This phenomenon is in alignment with the human population in which there are people who develop depressive phenotype (susceptible) and others who do not (resilient) after trauma exposure. It is assumed that those individuals exhibit various neural mechanisms that underlie pathological synaptic plasticity (depressive behavior) and those actually associated with stress adaptation (resilience). Here, using a multidisciplinary approach we characterized the excitatory synapses in the hippocampus to assess the molecular fingerprint of stress resilience in an animal model of depressive-like behavior. Our results showed that chronic unpredictable stress leads to the development of anhedonic and resilient behavior associated with various glutamatergic neurotransmission, structural remodeling of dendritic spines, and protein profile. Moreover, the distinction between anhedonic and resilient animals enables to propose a new posttranslational mechanism underlying the behavioral switch in stress response that appears to be a promising tool for further pharmacological treatments.

Daria Guseva (Institute of Nutritional Medicine, University of Hohenheim, Germany)

Stress, Microbiota-Gut-Brain-Axis and Nutrition

The communication between the gut and the brain takes place in a variety of ways and plays a crucial role in neurological and psychiatric diseases, particularly in stress-related disorders. This communication is known as the "gut-brain axis", and it is often named as a "microbiota-gut-brain axis". Signal transduction occurs in three overlapping pathways: neuronal, endocrine, and immunological. In this context, the gut microbiota can directly influence brain function by producing both neurotransmitters and neuropeptides, and by causing the production of neuroactive metabolites. However, the mechanisms of this communication are not clear.
Changes of microbial composition and their metabolites are highly regulated by dietary intake. This suggests a pivotal role of the nutritional style in functionality of the gut-brain-axis and its role in the onset of neurological diseases. Here, using the mouse model of high-fat and high-sugar diet, we investigated changes of brain plasticity and neuroinflammation, intestinal inflammation and intestinal barrier function associated with a dietary intake. Furthermore, we analyzed the gut microbial profile associated with stress in the chronic unpredictable stress (CUS) mouse model.
Results of our first experimental set indicate changes of a number of GABAergic as well as dopaminergic neurons by chronic consumption of different types of diet. In the second sets of experiments, we have found a significant shift of microbial compositions associated with stress-related depression. Our data suggest that the implication of dietary intake to reverse the gut microbial composition can prevent changes of the brain plasticity associated with the stress-related disorders.

Gregory Petrazzo (Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland)

Combination of Dasatinib and Quercetin improves cognitive abilities in aged Wistar rats

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Introduction. Neurons and other glial cells have the potential to acquire senescent characteristics that could lead to defect in neuronal plasticity and alteration of cognition which negatively impact quality-of-life of elders. Eliminating senescent cells that accumulates with age, using senolytics drugs, has proven to be effective in alleviating symptoms of aged-related diseases.
Hypothesis. Combination of Dasatinib and Quercetin senolytics (D+Q) might prevent cognitive decline observed in aged rats.
Objectives. Quantify systemic inflammation level since inflammaging is a key component of unhealthy aging. Assess synaptic plasticity in hippocampal structures that are principally involved in memory processing, spatial processing and navigation. Investigation epigenetic and senescence hallmarks to shed light on relevant molecular pathway affected by D+Q treatment.
Methods. Young (3-month-old) and naturally aged male Wistar rats (18-/22-month-old) were treated with D+Q for eight weeks and tested in the active allothetic place avoidance task. Arterious blood was collected to assess cytokines level. Fresh hippocampal slices were stained with DiI to analyze dendritic spine morphology. Epigenetic and senescence markers were quantified from fixed hippocampal slices or lysates.
Results. We confirmed the cognitive decline of aged rats compare to younger animals. We observed in aged but not young rats treated with D+Q a reduction in systemic inflammation and an alleviation of aged-related learning deficits and memory impairments associated with changes in synaptic plasticity and epigenetic but not senescence markers. Furthermore, D+Q treatment retains long lasting effects up to six weeks after treatment.
Conclusion. Our study brings new insights on the effects of D+Q senolytics in alleviating age- associated cognitive dysfunctions.

Błażej Ruszczycki (Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland)

Visualizing of Topologically Associated Domains using Three-Dimensional Electron Microscopy In Situ Hybridization

Organization of chromatin in the interphase nucleus still remains elusive. Understanding of the way in which a two-meter strand DNA is packaged in ~ 10 micrometers-size nucleus will allow us to better understand not only organization of the basic structural units but also shed light on the functional aspects like regulation of gene expression and causes of genetic diseases. However, the resolution of light microscopy is not enough to study chromatin fibers with a diameter about 5-30 nm. Recent superresolution methods such as localization microscopy achieve lateral resolution as low as 10–30 nm, and moreover do not have the sufficient imaging depth to visualize the stranded DNA fragment. As an alternative for them, we developed a method based on the three dimensional electron microscopy with lateral resolution down to 5 nm. Our high resolution approach -3D-EM-ISH combines three dimensional electron microscopy (3D-EM) and DNA in situ hybridization (ISH). We used 3D-EM-ISH to probe the structure of chromatin domains revealed by ChIA-PET (Chromatin Interaction Analysis by Paired-End Tag Sequencing) – the technique to study spatial chromatin interactions. We propose our method as a tool to study how these domains - basic structural and functional units of chromatin are organized in single cells, and present their structure and cell to cell variability. We discuss reconstruction and quantification of the image, in particular the detection and segmentation 3D structures of topologically associating domains (TADs) in electron microscopy images. TADs are suspected to be fundamental units of three-dimensional genome organization.

Elise Tse (Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland)

Generation of human neurons from induced pluripotent stem cells for potential screening of drug candidate for neuropsychiatric diseases

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In-depth understanding of and novel effective treatments for neuropsychiatric diseases have been lagged over decades due to a huge gap in the development of an appropriate and faithful disease model. In this study, hoping to overcome the limitations of animal models or post-mortem patient brain tissues; to address the existence of neuroanatomical differences between humans and animals; and to reveal dynamic cellular changes in disease mechanisms, the technology of induced pluripotent stem cells (iPSCs) or iPSC-derived neurons have been applied to generate a human neuronal model which is hoped to serve as a starting point for answering the mechanistic questions and screening novel drug targets.
A commercially available Caucasian male iPSC line has been used in the study. Firstly, neural progenitor cells (NPCs) were differentiated via dual SMAD inhibition from iPSCs. The NPCs were then differentiated into neural precursors which eventually gave rise to neurons under further maturation. The NPCs and neural precursors expressed neural stem cells markers such as Nestin, Pax6. The resulting neurons expressed neuronal markers such as βIII-Tubulin, MAP2. Spine-like protrusions could be observed in neurons undergoing 1-month maturation.
The establishment of neurons from human iPSC will pave the way for downstream experiments to study or evaluate, for examples, post-translational modifications of protein in human neurons in stress-related conditions and treatment of novel drug candidates.

Agata Pytyś (Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland)

Efficacy of selected inhibitors of protein S-palmitoylation in synaptic plasticity

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Learning and memory require functional modification of neuronal networks through re-organization of existing synapses and modification of their efficacy. The rapid, neuron-activity induced posttranslational modifications of synaptic proteins have been suggested to play a key role in this process. S-palmitoylation (S-PALM), the reversible attachment of the fatty acid palmitate to cysteine residues may affect membrane localization or conformation of the modified protein. Therefore, S-PALM has been suggested to play an important role in protein trafficking and localization. However, its precise role in synaptic plasticity remains elusive. The lack of pharmacological control of S-PALM in nervous tissue significantly limits the research in this area. There, we have employed Acyl-Biotin Exchange (ABE) assay and investigated the changes in S-PALM profiles following exposition of brain slices to commercial and non-commercial inhibitors of S-PALM as well as hydroxylamine derivative that cleaves thioester linkage with high potency and specificity in tissue extracts. We have compared the efficacy of S-PALM inhibition in synaptoneurosomes of rat brains or homogenates of cultured hippocampal neurons following enhanced neuronal activity. In addition, the dose-dependent neurotoxicity of investigated compounds was tested.
Research funded by the polish National Science Center (grant number 2017/26/E/NZ4/00637 and 2019/34/E/NZ4/00387).

Marta Czarnota-Bojarska (Nencki Institute of Experimental Biology Polish Academy of Sciences, Poland)

SNO-TRAP synthesis and possible applications

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Posttranslational modifications (PTMs) influence many protein functions. S-nitrosylation and S-palmitoylation are PTMs of proteins involving the addition of NO group or palmitic acid, respectively, to the cysteine thiols. Crosstalk between those two posttranslational modifications regulates signaling pathways. Changes in the pattern of any of those PTMs can lead to neurodegenerative and neuropsychiatric disorders, such as Alzheimer's Disease. Despite the biological significance of S-nitrosylation, for many years there was no precise method of detecting this PTM. The only procedure of labelling S-nitrosylated cysteines was biotin-switch technique (BST). The main disadvantage of BST is that false positives can occur because of incomplete blocking of free cysteine thiols.
Finding the method of direct labelling of S-nitrosylated cysteines was the challenge of modern neuroscience, which led to the development of SNO-TRAP probe . SNOTRAP (SNOtrapping by triaryl phosphine) is a new technique that allows improved identification of S-nitrosylated proteins. This method uses phosphine-based chemical probes which react with SNO groups and allow unequivocal confirmation of S-nitrosylated cysteines. I will show synthesis of the SNOTRAP probe and its possible applications.