## 15TH INTERNATIONAL CONFERENCE OF THE LITHUANIAN NEUROSCIENCE ASSOCIATION

### 24th November 2023, Kaunas, Lithuania

Centre for the Advanced Pharmaceutical and Health Technologies Lithuanian University of Health Sciences Hall A-203, Sukileliu av. 13, Kaunas, Lithuania









15th International Conference of the Lithuanian Neuroscience Association "Neurodiversity: from Theory through Artificial Intelligence to Clinical Practice"

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### 15th International Conference of the Lithuanian Neuroscience Association "Neurodiversity: from Theory through Artificial Intelligence to Clinical Practice"

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### **PROGRAM**

9.00-10.00	Registration. Coffee/Tea
10.00–10.10	<b>Opening and welcome</b> Prof. OSVALDAS RUKŠĖNAS, President of the Lithuanian Neuroscience Association Prof. VAIVA LESAUSKAITĖ, Vice-rector for Research, Lithuanian University of Health Sciences Prof. ARIMANTAS TAMAŠAUSKAS, Director, Neuroscience Institute, Lithuanian University of Health Sciences Dr. RIMA NAGINIENĖ, Neuroscience Institute, Lithuanian University of Health Sciences
	<b>I session. Artificial Intelligence and Theoretical Neuroscience in Precision Medicine</b> Chair Prof. <b>AUŠRA SAUDARGIENĖ</b> , Neuroscience Institute, Lithuanian University of Health Sciences / Vytautas Magnus University, Lithuania
10.10-10.50	The Virtual Brain Models: Linking Theory and Data Towards Understanding Pathophysiological Processes Prof. PIERPAOLO SORRENTINO, Institute of System Neuroscience, Aix-Marseille University, France
10.50–11.30	<b>Precision Neurostimulation of Spinal Cord in Spinal Injury: a Computational Approach</b> Dr. ANDREAS ROWALD, Friedrich-Alexander University Erlangen-Nurnberh, Germany
11.30–11.45	<b>Photoplethysmography, Wearable Devices, and Selected Applications</b> Prof. <b>VAIDOTAS MAROZAS</b> , <i>Biomedical Engineering Institute, Kaunas University of Technology, Lithuania</i>
11.45-12.00	Meeting of the members of Lithuanian Neuroscience Association
12.00-13.00	Lunch
13.00-14.00	Poster session
	<b>II session. Neurodegenerative Disorders</b> Chair – prof. <b>RAMUNĖ MORKŪNIENĖ</b> , <i>Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania</i>
14.00–14.40	DCLK3, Neuronal Kinase with Likely Many Roles: From Huntington's Disease to Psychiatric Disorders Dr. EMMANUEL BROUILLET, Institute of Biology (INSB) CNRS, France
14.40-15.00	Biomarkers in Amyotrophic Lateral Sclerosis EVELINA GRUŠAUSKIENĖ, Faculty of Medicine, Lithuanian University of Health Sciences, Lithuania
15.00-15.20	How Viral Inflammation Contributes to Neurodegeneration Dr. SILVIJA JANKEVIČIŪTĖ, Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania
15.20-15.50	Coffee/Tea. Poster session
	III session. Genetic Aspects of the Nervous System Diseases Chair – dr. PAULINA VAITKIENĖ, Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania
15.50-16.10	<b>RNA Modifications in Tumors – the New Black in Gene Regulation</b> Dr. GIEDRIUS STEPONAITIS, Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania
16.10–16.30	Interplay between Metabolic and Epigenetic Factors in Transmission of Neuropsychiatric Disease Risk Dr. ALI JAWAID, Nencki-EMBL BRAINCITY Center of Excellence for Neural Plasticity and Brain Disorders, Nencki Institute of Experimental Biology, Warsaw, Poland
16.30–16.45	Visual Information Processing in Cortex and a Potential Link to Brain Disorders Dr. GYTIS BARANAUSKAS, Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania
16.45-17.00	Concluding remarks, discussions, awards
17.00-19.00	Closing party

## Oral presentations



### The Virtual Brain Models: Linking Theory and Data Towards Understanding Pathophysiological Processes

#### Prof. Pierpaolo Sorrentino

Institute of System Neuroscience, Aix-Marseille University, France ppsorrentino@gmail.com

The primary approach in brain research involves merging the perspectives of network dynamics and graph theory metrics. The network dynamics framework is rooted in the idea that the brain's architecture significantly influences its neurophysiological activities. Consequently, when investigating the brain's large-scale dynamics, researchers often depict it as a network composed of anatomically connected regions, each governed by its inherent dynamics and by the activities of other regions.

This approach has led to various modeling studies demonstrating that the neuroanatomical constraints and the local dynamics play a pivotal role in shaping the brain's functionality during the resting state. This has proven a viable avenue of investigation for diseases such as epilepsy. A new frontier in clinical applications is the individualized large-scale computational modeling of brain pathology dynamics.

However, in order to deploy personalized models in clinical practice, one needs to find reliable read-outs of the pathophysiological processes. Research has predominantly focused on functional connectivity (FC), which quantifies the statistical dependencies among signals produced by distant brain regions.

However, more recently, it has been established that FC is not static but exhibits non-stationarity, revealing complex dynamics.

This talk will provide an overview of the interplay between large-scale brain models and multimodal data. Firstly, the talk will provide some examples of how to interrogate large-scale neuronal data, with respect to the features to capture dynamics (i.e. MEG/EEG), in order to design valid and reliable biomarkers. Then, the talk will move on to demonstrate how to use large-scale brain models and statistical analysis to infer potential microscopic mechanisms underpinning the observed changes on the large scale.

## Precision Neurostimulation of Spinal Cord in Spinal Injury: a Computational Approach

#### Dr. Andreas Rowald

Friedrich-Alexander University Erlangen-Nurnberh, Germany andreas.rowald@fau.de

Efficient identification of effective, safe, and personalized spinal cord stimulation parameters is critical due to the growing clinical applications, complex technologies, and pathophysiological variations among individuals. In turn, this approach offers cost-, time-, and risk-limited therapeutic options for neurological disorders. Here, we present a computational method using multi-scale digital twin models for therapy optimization and clinical decision support of spinal cord stimulation in the use-case of lower limb motor recovery after spinal cord injury. Our computational method informed the design of a specialized electrode lead and guided its neurosurgical placement, leading to three individuals with complete sensorimotor paralysis being able to stand, walk, cycle, swim, and control trunk movements with spinal cord stimulation paradigms identified within a single day, as opposed to several weeks in previous attempts.

## Photoplethysmography, Wearable Devices, and Selected Applications

#### Prof. Vaidotas Marozas

Biomedical Engineering Institute, Kaunas University of Technology, Lithuania vaidotas.marozas@ktu.lt

The lecture will provide a brief history of wearables and photoplethysmography (PPG), discuss hypotheses related to the origins of PPG, explore PPG sensors, and introduce the novel integrated wearable device KTU\_watch. This device has already been utilized in numerous research projects. Although PPG signals are convenient to register, they pose challenges due to signal quality and artifacts. Nevertheless, these signals show promise for long-term unobtrusive monitoring of cardiac arrhythmia, blood pressure, pain, and psychophysiological stress.

## DCLK3, Neuronal Kinase with Likely Many Roles: From Huntington's Disease to Psychiatric Disorders

Lucie de Longprez<sup>1</sup>, Marie-Claude Gaillard<sup>1</sup>, Charles Decraene<sup>2</sup>, Celine Keime<sup>3,4,5,6</sup>, Mathilde Louça<sup>1</sup>, Carole Jan<sup>1</sup>, Géraldine Liot<sup>1</sup>, Julien Mitja<sup>1</sup>, Nachiket Nadkarni<sup>1</sup>, Philippe Hantraye<sup>1</sup>, Fabien Bertaux<sup>7</sup>, Marie Norbert<sup>7</sup>, Gilles Bonvento<sup>1</sup>, Julien Flament<sup>1</sup>, Marc Dhenain<sup>1</sup>, Christian Neri<sup>8</sup>, Alexis Bemelmans<sup>1</sup>, Karine Mérienne<sup>2</sup>, Karine Cambon<sup>1</sup>, Emmanuel Brouillet <sup>1,8</sup>

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More than 500 kinases that likely play crucial roles in cell signaling are encoded in the human genome. Approximately 250 kinases are expressed in the brain. Only a minority has been studied experimentally. Thus, for a majority of them, the roles of kinases in the brain remain largely unknown.

We will present our studies related to DCLK3 (Doublecortin-like kinase 3) kinase. DCLK3 is expressed in the cytoplasm and nucleus of neurons. DCLK3 is preferentially expressed in GABAergic projection neurons of the striatum, and striatal levels of DCLK3 mRNA are markedly reduced in patients with Huntington's disease (HD), an inherited neurodegenerative disorder caused by abnormal expansion of CAG repeats in the huntingtin (HTT) gene. Genetic mouse models of HD also showed reduced expression of DCLK3 . Loss of DCLK3 function in adult mice renders striatal neurons more vulnerable to mutant HTT, while increased DCLK3 expression produces neuroprotection via its kinase activity 1. On the other hand, because recent studies on large cohorts of patients suggest that DCLK3 polymorphisms could be risk factors for psychiatric diseases illnesses. Thus, we sought to further explore the roles of DCLK3 in the brain through the study of Dclk3 knockout and conditional knockdown mouse models that we generated. We will present the recent multidisciplinary characterization of these new models, which suggests that DCLK3 is involved in stress-like

behavior, and memory. Transcriptomic analyses of the hippocampus of conditional Dclk3 knockdown mice also suggest that DCLK3 regulates key molecular players required for synaptic plasticity and memory processes.

**Acknowledgements.** We thank Julien Mitja and Patrick Hery for their technical assistance in animal experiments.

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### **Biomarkers in Amyotrophic Lateral Sclerosis**

#### Evelina Grušauskienė, Brigita Gusarovienė, Daiva Garšvienė, Daiva Rastenytė

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Amyotrophic lateral sclerosis (ALS) is neurodegenerative disorder leading to progressive muscle weakness, respiratory dysfunction and death. Despite extensive research of exposome it is still not understood which persons might be affected, what might be the course of the disease.

We performed neuromuscular ultrasound (US) for 23 patients with ALS and 33 healthy controls (HC). Nerve cross sectional area (CSA) and echogenicity was evaluated. Mean hyperechoic fraction of white was calculated as a percentage. Muscle echogenicity was evaluated using Heckmatt scale (HS). Disease severity was evaluated using ALS-FRS-R scale, serum's concentration of phosphorylated neurofilament heavy chain (pNfH) was analysed.

Mean age of patients with ALS was 58.3 (SD 11.4) years, of control group - 53.5 (SD 11.8) years. Increased echogenicity was found in the anterior tibial (AT) (85%), quadriceps femoris (93%), 1st dorsal interosseous (1stDO) (63%), and submental (56%) muscles of ALS patients.

Biceps brachii muscle strength of the patients with muscle echogenicity assigned to 1 point by HS was bigger compared to those with muscle echogenicity of 2 points by HS (p<0,001). Also AT muscle strength was bigger of the patients with muscle echogenicity of 1 point by HC compared to those of 3 points by HS (p<0.001).

ALS-FRS-R scale score was higher in patients with right 1stDO muscle echogenicity of 1 point compared to those with 3 points (p<0.04).

The upper trunk (UT) of the right brachial plexus had a median fraction of white of 34.6% in ALS patients, compared to 17.8% in HC, (p=0.01). Also the left ulnar nerve in the midline of the forearm (FA) had median fraction of white of 66.2% while corresponding fraction in HC was 77.9%, (p=0.03).

Median CSA of the UT of the right brachial plexus was 3.6 mm2 in ALS patients and 5 mm2 in HC (p=0.01).

Mild but significant correlation was observed between lower ALS-FRS-R scale score and lower echogenicity of the left UN (r=0.44, p=0.047).

Median concentration of pNfH in ALS patients was 43.4 pg/ml, and did not differ between bulbar-onset and limb-onset ALS groups (41.6 pg/ml and 42.5 pg/ml, respectively, (p=0.78)).

Our results show that patients with hyperechoic muscles had less strength. Size and US echogenicity changes of peripheral nerves were associated with worse physical function. No differences were found in pNfH concentration according to disease phenotype. Further studies are needed with extended cohort.

## How Viral Inflammation Contributes to Neurodegeneration

#### Dr. Silvija Jankeviciute

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An expanding body of evidence indicates a potential link between neuroinflammatory reactions prompted directly or indirectly by viral infections and the onset or advancement of neurodegenerative diseases. Viral infections can induce innate immune response or cause the infiltration of peripheral immune cells into the central nervous system, consequently leading to neuroinflammation. Moreover, viral infections can induce pathological changes of amyloidogenic proteins involved in pathogenesis of neurodegenerative diseases. In case of Parkinson's disease, the second most common neurodegenerative disorder, pathological changes induced by viruses may trigger an inflammatory response and result in both – direct activation of glial cells and increased  $\alpha$ -synuclein expression and aggregation leading to neurodegeneration. However, the exact mechanism of virus-induced neuronal loss remains unclear. In the talk, S. Jankeviciute will discuss a possible mechanism how RNA-virus mimetics can cause microglia-mediated neuronal loss and synergize with  $\alpha$ -synuclein.

**Funding.** This work was supported by the Research Council of Lithuania, Researcher Groups projects No S-MIP-23-98 (APNEVIR).

## RNA Modifications in Tumors – the New Black in Gene Regulation

Giedrius Steponaitis, Rugile Dragunaite, Rytis Stakaitis, Paulina Vaitkiene, Arimantas Tamasauskas, Daina Skiriute

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Recently, transcriptome-wide studies have characterized different types of chemical modifications of coding and non-coding RNAs. Today it is evident that RNA modifications are highly prevalent, dynamic and reversible, which makes them critical component of posttranscriptional gene regulatory landscape in biological processes and diseases 1. Epitranscriptomic modifications can alter RNA molecule local charge, base-pairing potential, secondary-structure and RNA- protein interactions which in turn shape gene expression. Among over 100 different modifications in RNA, N6-methyladenosine (m6A) is the most prevalent epigenetic modification of mRNA and noncoding RNAs in eukaryotes 2. Global profiling of epitranscriptome m6A modifications in long non-coding RNAs (lncRNAs) of glioblastomas (GBs), low grade gliomas (LGGs), glioma stem cells (GSCs), and neural stem cells (NSCs) uncovers another biological layer of gene regulation which may reveal unexpected and clinically relevant molecular markers for glioma diagnostics and targeted therapy. The lncRNAs m6A profiling of GSCs, GBs, LGGs and non-tumorous brain tissues was performed applying two different techniques - MeRIP-seq and direct RNA sequencing on nanopore (Oxford Nanopore Technologies). The first part of the study revealed ~20.7% of all identified lncRNA transcripts had at least one m6A peak indicating m6a methylation importance for lncRNA functioning in gliomas. GSCs had slightly more m6A methylated lncRNAs as compared to conventional GBM cell line - 20.28% vs 18.78% and m6A modification rate negatively correlated with lncRNAs expression level. Thus, we speculated that m6A modification can stabilize lncRNAs and lncRNA may overcome the need for abundance in molecular numbers 3. In the next part of the study, significantly higher m6A levels of lncRNAs and mRNAs in LGGs as compared to GBs were found, indicating that more aggressive brain tumors are getting rid of m6As. Nevertheless, we haven't found the link between m6A machinery and m6A levels of lncRNAs in human brain tumors. Taking all together the very pilot study revealed m6A profile differences between different malignancies of brain tumors and cells with stemness properties that may provide new insights regarding the association between m6A methylation of lncRNAs, its functioning, and gliomas.

## Interplay between Metabolic and Epigenetic Factors in Transmission of Neuropsychiatric Disease Risk

#### Dr. Ali Jawaid

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Childhood trauma is an important risk factor for psychiatric and physical ailments during adulthood. Emerging evidence from rodent studies suggests that some behavioral and metabolic symptoms of childhood trauma are transmissible across generations. However, the translational implications of this novel concept are in the preliminary stages. This talk entails a systematic examination of small RNAs in the serum, sperm, and milk samples collected from ethnically diverse human trauma cohorts with an overarching aim to identify the molecular underpinnings of the long-term effects and transmission of trauma symptoms. Small RNA sequencing (sRNA-seq) followed by RT-qPCR assays were performed to identify and validate miRNA changes in the serum of children with recent trauma in the form of paternal loss and maternal separation (PLMS), in the sperm of adult men with a history of complex trauma before the age of 17, and in the milk of lactating mothers with history of adverse childhood experiences. Pathway analysis of the differentially expressed miRNAs in these diverse samples suggest a potential role for cholesterol signaling in the long-term propagation and transmission of trauma. Notably, miR-223-3p, which was similarly upregulated in the blood, sperm, and milk samples from these trauma cohorts targets SR-B1: the receptor for high-density lipoproteins (HDLs) and is implicated in cholesterol biosynthesis. Guided by these results, our current efforts are focused on modeling the role of lipids and lipid-associated factors in the long-term effects and transmission of childhood trauma via ethologically relevant mouse models and ex vivo approaches.

## Visual Information Processing in Cortex and a Potential Link to Brain Disorders

#### Dr. Gytis Baranauskas

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In visual cortex principal cells, the pyramidal neurons, can activate inhibitory interneurons that in turn control the visual response gain of other pyramidal neurons via suppression of visual responses. By employing optogenetic stimulation we show that both pyramidal and interneuron stimulation reduces receptive field (RF) area and increases signal-to-noise ratio (SNR) for such visual responses. We also show that such an increase in SNR increases information about visual stimuli that is encoded by action potentials. Since all sensory inputs will activate pyramidal neurons, it is concluded that any activation of visual cortex, be due to sensory inputs or state of wakefulness may enhance visual information processing. Since inhibitory neuron function is affected in brain injury and several brain disorders, these results may help to better understand the pathogenesis of brain dysfunction.

## Poster presentations



## Tumour Treating Fields (TTFields) effects on cerebEND cell culture as blood-brain barrier model

Aiste Giniunaite<sup>1</sup>, Almuth Kessler<sup>1</sup>, Ellaine Salvador<sup>1</sup>, Julia Hörmann<sup>1</sup>, Dominik Domröse<sup>1</sup>, Malgorzata Burek<sup>2</sup>, Ralf-Ingo Ernestus<sup>1</sup>, Mario Löhr<sup>1</sup>, Carola Förster<sup>2</sup>, Carsten Hagemann<sup>1</sup>

TTFields are an FDA approved treatment for glioblastoma (GBM) as well as pleural mesothelioma. They are low intensity (1-3 V/cm), intermediate frequency (100-500 kHz) alternating electric fields that are applied therapeutically from two directions by a portable, non-invasive device. TTFields disrupt proteins that are essential to cell division. Thereby TTFields inhibit proliferation and spreading of cancer cells. The blood-brain-barrier (BBB) is a characteristic of the brain vasculature that shields the brain from toxic substances circulating in the blood. However, it is also the limiting factor for a multitude of highly effective chemotherapeutic drugs, which are prevented by the BBB to reach their targets within the brain. We showed that application of TTFields at 100 kHz temporarily opens the BBB by Rho-ROCK signalling pathway mediated phosphorylation of the tight junction protein claudin-5 and its subsequent internalization. Cessation of TTFields treatment led to complete BBB recovery after 72 to 96 hours. This effect was repeatable. However, switching TTFields from 100 kHz for initial opening of the BBB to the GBM-treatment effective 200 kHz let the BBB to remain open during the whole treatment duration. Controlled opening of the BBB could allow for increased drug concentrations within the CNS, allowing BBBimpermeable drugs to be utilized for treatment of CNS diseases.

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## Influence of STAT4 rs7601754 gene polymorphism on the incidence of multiple sclerosis in Lithuania

### Akvile Bruzaite<sup>1</sup>, Greta Gedvilaite<sup>1</sup>, Renata Balnyte<sup>2</sup>, Rasa Liutkeviciene<sup>1</sup>

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**Introduction:** Multiple sclerosis (MS) is a chronic autoimmune disease that affects the central nervous system (CNS) and is described by demyelination, inflammation, gliosis, and the loss of neurons. MS is characterized by a dysregulation of the immune system that leads to infiltration of the CNS by immune cells. Since MS is related to immune system dysregulation, it may be associated with Signal Transducer And Activator Of Transcription 4 (STAT4). Consequently, STAT4 is a critical mediator of the immune response, and any alteration in STAT4 expression or activity can impair the response and function of the regular immune system, potentially leading to autoimmune disease. MS has a multifactorial etiology, which involves both hereditary and environmental factors. The interaction between genetic and environmental factors in MS remains an active area of research to find new targets for the prevention and treatment of the disease. Further research is needed to elucidate the role of the *STAT4* gene in MS and to determine whether targeting this gene could be a potential therapeutic strategy for the disease.

**Materials and methods:** The study involved 200 patients with MS and 200 healthy controls as a reference group. Patients were screened using the 2017 diagnostic criteria, which included positive oligoclonal bands, typical demyelinating lesions on magnetic resonance imaging (MRI) scans of the brain and spinal cord (according to the MAGNIMS criteria), and clinical symptoms/relapses. Deoxyribonucleic acid (DNA) was extracted from peripheral venous blood using DNA salting-out method. Genotyping of *STAT4* rs7601754 was performed by real-time polymerase chain reaction (RT-PCR).

**Results:** According to the most suitable dominant model, the *STAT4* rs7601754 was associated with a 1.9-fold increased odds of MS development (OR=1.912; 95% CI: 1.237-2.954; p=0.004). Moreover, each A allele was associated with 1.7-fold increased odds of MS development in the additive model (OR=1.732; 95%. CI: 1.193-2.516; p=0.004). AA genotype and A allele were statistically significantly less frequent in MS patients compared to the control group individuals (63% vs. 76.5%, p=0.003, 79% vs. 87%, p=0.003, respectively).

**Conclusion:** In conclusion, our research indicates the potential impact of *STAT4* rs7601754 on the occurrence of MS. The *STAT4* rs7601754 AA genotype and A allele were associated with increased odds of MS development.

## Supporting effect of WJ-MSC and NSC on tissue restoration after ischemic brain injury – ex vivo study

## Aleksandra Bzinkowska<sup>1</sup>, Klaudia Radoszkiewicz<sup>1</sup>, Daniela Ferrari<sup>2</sup>, Anna Sarnowska<sup>1</sup>

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During ischemic stroke, there is a cascade of pathological changes related to small vessel injury and the neurovascular unit (NVU) disintegration. The central nervous system has a limited ability to self-repair after damage; therefore, finding alternative treatments to promote recovery is crucial for stroke treatment. The mesenchymal stem/stromal cells (MSCs) can influence the regeneration of the damaged tissue, protect/support neural stem cells (NSCs) and endothelial progenitors (components of the neural stem cell niche) due to their therapeutic properties especially strong adjuvant capabilities. The goal of this research was to enrich the damaged tissue microenvironment with MSCs and MSC-derived biological activity molecules, thus supporting its regeneration processes. In this project, we co-cultured MSCs derived from Wharton's jelly (WJ-MSCs) with injured neural tissue (oxygen- glucose deprived organotypic hippocampal slices) and neural stem cells. We investigated bilateral interactions between MSCs, injury tissue and NSCs. Therefore, not only we verified the changes in paracrine activity of WJ-MSCs cultured in the presence of the injured tissue and inserted NSCs but also we assessed the neuroprotection potential both of cell populations. When it comes to WJ-MSCs which had been cultured with the injured slices, we observed an increased secretion of growth factors such as EGF and GDNF. Moreover, a high secretion level of the factors that influence vascular remodeling (eg. CCL2, VEGF-C) was observed. An increase in neural gene expression level was also observed. The presence of NSCs enhanced the secretory properties of MSCs - they secreted higher levels of selected factors when compared to MSCs cultured alone. Both cell populations showed a neuroprotective effect in the ex vivo model. In conclusion, the presence of the injured brain tissue stimulated MSCs to secrete higher levels of selected factors. WJ-MSCs can improve vascularization after ischemic stroke injury. NSC and WJ-MSC have the neuroprotective potential for tissue after ischemia injury and potentiate mutual effect. This study brings us closer to understanding the role of MSCs as support for the cell niche in tissue regeneration.

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## Perceived size of objects with motion-defined contours

#### Algis Bertulis, Arūnas Bielevičius, Tadas Surkys, Niloy Talukder

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An increase in the relative size of visual objects (the illusion of expansion) was constantly observed in psychophysical experiments with stimuli that differed in shape, size, and orientation and were outlined by a spatial contrast in luminance, color, or texture, also defined by lines, perceptual grouping, and subjective (illusory). The strength of the illusion monotonously increased from a minimum to about 20 arc min with an increase in the length of the stimuli from 25 to 225 arc min for various contours tested, except the Kanizsa type. The latter caused twice weaker effects: the maximum value approached 10 arc min. Thus, subjective contours provided a weaker spatial signal than real ones. The contour may not be a uniform condition for the illusion. To test the prediction, the following type of contour was taken for experiments - defined by motion. Borders of the rectangular-shaped stimulus were formed by random-dot patterns drifting on a stationary random-dot background. In the absence of drift, the rectangle became invisible. The data obtained showed intriguing news - the absence of overestimation of the relative length of the stimulus in the judgments. Even more, all subjects produced gentle errors of underestimation of the distance. The average value lowered from about zero to -5 arc min with increasing the stimulus length from 25 to 225 arc min. The present result agreed to some extent with previous literature data demonstrating changes in perceived size for moving objects, e.g.: grating motion's apparent position shifted in the direction of motion, and moving objects seemed smaller. Taken together, the present result showed an exception to the general rule - the contours of motion do not create the illusion of expansion.

## Multiple sclerosis: may IL-9 SNP play a role in the disease occurrence in Lithuanian population?

### Alvita Vilkevičiūtė<sup>1</sup>, Kriste Kaikaryte<sup>1</sup>, Greta Gedvilaite<sup>1</sup>, Renata Balnyte<sup>2</sup>, Rasa Liutkeviciene<sup>1</sup>

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**Background:** Multiple sclerosis (MS) is a persistent inflammatory autoimmune disorder affecting the central nervous system (CNS) (1). Although the precise etiology of multiple sclerosis remains unknown, we hypothesized that genetic factors involved in inflammatory responses could contribute significantly, as observed in the pathogenesis of various diseases. This investigation focuses on examining the association between a single nucleotide polymorphism (SNP), specifically IL-9 rs2069885, and the incidence of MS in the Lithuanian population.

**Methods:** In current study, we carried out genotyping of IL-9 rs2069885 in 318 subjects (218 control group subjects and 100 MS patients) and analyzed possible associations between selected SNP and MS development. SNP was genotyped using TaqMan SNP genotyping assays by real-time PCR method, and the statistical analysis was performed using the SPSS/W 29.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA).

**Results:** Statistical analysis revealed that IL-9 rs2069885 genotype (GG, GA and AA) distribution was significantly different comparing patients with MS and control group (25 %, 70 % and 5 % vs. 66.5 %, 30.3 % and 3.2 %, p<0.001). Binomial logistic regression showed that IL-9 rs2069885 GA genotype is associated with the higher odds of MS development under the codominant (OR=6.152; 95% CI:3.579–10.572, p<0.001) and overdominant (OR=0.53; 95% CI:0.30–0.95, p=0.035) models. Moreover, GA and AA genotypes together showed the similarly high chances of MS occurrence under the dominant genetic model (OR=5.959; 95% CI:3.497–10.153, p<0.001) and the genotype AA under the codominant model (OR=4.143; 95% CI:1.219–14.083, p=0.023).

**Conclusion:** Our study revealed that IL-9 rs2069885 might play a role in MS development in Lithuanian population.

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## Perceived relative size of objects with moving surface elements

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The motion-defined contours did not create the illusion of expansion. Conversely, a slight decrease in the relative length was recorded in experiments with a comparison of the reference and test distances. This physiological feature of motion contours distinguished them from other types of edges: outlined by contrast in luminance, color, and texture or determined by lines, perceptual grouping, and imaginary boundaries. According to the literature data, moving objects appeared smaller than static ones, and the decrease in size was greater for faster speeds. But whether this rule of relative size reduction also applies to objects that do not move on the background, and only their contours are determined by moving elements - this was the purpose of this study. In the experimental presentations, random-spot patterns rectangular in shape were sliding top dawn on a stationary random-spot background. Different speeds of surface movement were used: 0.25°, 1°, 2°, 3°, 4°, and 5° per second. Subjects judged the width of the referential rectangle by using the adjustments method. The values of the perceived relative length as functions of the stimulus size were established. The size expansion signs were absent in the data obtained. The average error value reached -3 arc min, and one-on-one dropped to -6 or -10 arc min. A barely noticeable decrease in perceived relative width with increasing stimulus size was present at all speeds. However, it cannot be said with certainty that the speed of internal movement has almost no effect on the perceived relative size. Random spot texture patterns have a wide range of spatial frequencies and can sum up different contributions at different movement speeds. Further studies are recommended. Frequency mixing can be avoided by using sinusoidal gratings.

## Mean-field Population Model for the Basal Ganglia

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Parkinson's disease (PD), a prevalent neurodegenerative condition, is characterized by motor symptoms linked to dopamine deficits and pathological oscillations in the basal ganglia (BG) neurons within the  $\beta$  frequency range. This study leverages dynamical systems theory to develop a comprehensive model of the BG in PD, integrating key neural populations and their interactions. Building on advances in modeling heterogeneous neural networks with quadratic integrate-and-fire (QIF) neurons [1], we present a full BG network model. This includes the subthalamic nucleus (STN), globus pallidus externus/internus (GPe/GPi), striatum, and thalamus, each represented by a two-dimensional system of firing rate differential equations. The model captures the direct, indirect, and hyper-direct pathways, integrating dopaminergic inputs from the substantia nigra and cortical excitations. Our analysis focuses on the impact of high-frequency stimulation (HFS) on network dynamics. Applying HFS to STN and GPi, we identify parameter regimes in which network synchronization can be effectively suppressed. This intervention is modeled as an increase in the excitability parameter of the targeted neural populations, leading to a Hopf bifurcation that stabilizes the network's resting state and terminates pathological oscillations [2]. Additionally, we investigate the effects of cortical inputs and reduced dopaminergic input from the substantia nigra on BG dynamics. Our findings reveal that cortical inputs significantly influence the model's behavior, while a decrease in dopaminergic input correlates with the emergence of synchronized collective dynamics, mimicking PD pathology. This model's strength lies in its ability to seamlessly integrate and modify various neural populations, offering flexibility in network topology and enabling detailed analysis of both internal dynamics and responses to external stimulation. The primary limitation is its simplified representation of BG dynamics, which may not fully reflect in vivo complexities. Future validation against in vivo data is crucial for its applicability to Parkinson's disease. Moreover, the model's handling of cortical inputs is overly simplistic, necessitating more detailed future exploration to accurately capture their complex interactions with the basal ganglia.

1. https://doi.org/10.1103/PhysRevX.5.021028

2. https://doi.org/10.1103/PhysRevE.104.014203

# Impaired memory storage and recall in a hippocampal CA1 network in early Alzheimer's disease

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Alzheimer's Disease (AD) affects millions of individuals, and is one of the main causes of dementia and neurodegeneration worldwide. No significant progress has been made in the treatment of this condition. The molecular mechanisms leading to neurodegeneration in AD are still far from being clear. Alterations in the Amyloid Precursor Protein (APP) processing and clearance have been observed in early stages of AD. Increased levels of specific APP fragments, such as Amyloid  $\beta$  peptide (A $\beta$ ) and Amyloid APP Intra-Cellular Domain (AICD), are believed to play an important role in impairment of learning and memory in AD (Opazo et al, Cell Reports 2018; Pousinha et al, Elife 2017). We present a computational study on the effects of the Aβ-induced alterations in synaptic plasticity at a network level. We use a hippocampal CA1-CA3 network which consists of 100 CA1 pyramidal neurons with inhibitory interneurons, medial septum inputs, entorhinal cortex inputs and Schaffer collateral inputs from CA3 neurons. We employ a newly developed NMDAr-dependent voltage-based model of synaptic plasticity that implements the experimentally observed effects of increased levels of AICD and AB on long-term potentiation (LTP) and long-term depression (LTD) at CA1-CA3 synapses (Dainauskas et al, Front. Comput. Neurosci. 2023) We illustrate the influence of Aβ-induced alterations in synaptic plasticity on the pattern storage and recall ability of the network. We demonstrate that altered LTP impairs memory storage and recall in hippocampal CA1 network in AD, the process that can be prevented by pharmacological blockage of GluN2B-NMDA receptor. Computational modeling study allows integration of the complex effects of AD related peptides at molecular, synaptic, neuron and network levels, and explains the impaired memory formation and retrieval in the hippocampal networks in AD.

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## Integrity assay for messenger RNA in ribosome-depleted subcellular samples

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Traditionally, RNA integrity evaluation is based on ribosomal RNAs (rRNAs). In contrast, gene expression studies are usually focused on protein coding messenger RNAs (mRNAs). As rRNA and mRNA have significant structural and functional differences, the assumption that rRNA integrity properly represents mRNA integrity may not be accurate. Moreover, contrary to tissue RNA samples, subcellular preparations such as synaptosomes contain almost no rRNA, thus prohibiting the use of traditional rRNA-based methods to assess sample RNA integrity. We have developed a RT-qPCR based assay which estimates mRNA integrity by comparing the abundance of 3' and 5' mRNA fragments in a long constitutively expressed Pgk1 mRNA. The assay was tested and validated using plasmids with cloned 3' and 5' ends of the Pgk1 cDNA reflecting different ratios of 3' and 5' cDNA amplicons in the reverse transcribed RNA sample. The accuracy of integrity score calculation was ensured by integrating a mathematical correction of qPCR results to account for the amplification efficiency of different primer pairs. The 5':3' was used to quantify RNA degradation in heat degraded mouse and human brain tissue RNA as well as clinical RNA samples, which showed that different means of RNA degradation indeed affects mRNA and rRNA differently and therefore a 5':3' assay that evaluates mRNA integrity directly is more reliable. We then applied 3':5' assay to assess mRNA integrity in mouse synaptosomal preparations that lack rRNAs. We concluded that the 5'-3' assay can be used as a reliable and sensitive method to evaluate mRNA integrity in mouse and human brain tissue and subcellular preparations.

## Biomarkers of Parkinson's disease patients: analysis of magnetoencephalography data using Machine Learning methods

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**Introduction.** Parkinson's disease (PD), a progressive neurodegenerative disorder, stands as one of the most prevalent movement disorders. The precise etiology and mechanisms triggering this condition remain a subject of ongoing investigations.

Methods. This study aims at identification of the most informative features of Magnetoencephalography (MEG) data for discrimination between PD patients and healthy controls. The study included 40 individuals, 20 healthy subjects and 20 PD patients. MEG data was recorded using a 163-magnetometers MEG system. MEG data were preprocessed and source-reconstructed, based on the natives MRIs, according to the Automated Anatomical Labelling parcellation, yielding 116 time-series corresponding the 116 AAL regions. We filtered the source-reconstructed in five frequency bands: Delta (0.5-4 Hz), Theta (4-8 Hz), Alpha (8-12 Hz), Beta (12-30 Hz), Gamma (30-100 Hz) for each of 116 channels, 580 features in total. We selected 20 key features that best discriminated PD and healthy controls using the Minimum Redundancy Maximum Relevance (mRMR) feature selection method. Subsequent validation using Mann-Whitney test revealed that 18 out of these 20 features showed statistically significant differences between the two groups. The most informative features reflected MEG power spectra in Delta frequency band (channels 31, 27, 107), Theta frequency band (channels 95, 108, 115), Gamma frequency band (channels 98, 112, 115). The selected 20 features allowed the classification of participants into healthy control and PD groups, employing both statistical and machine learning algorithms. In total, eight machine learning methods (regularized binary logistic regression, decision tree classifier, linear discriminant analysis, naive Bayes classifier, random forest, kernel support vector machine, deep feed-forward neural network (DNN), feed-forward neural network-based autoencoder for anomaly detection (DNN-A)) were applied to build the classification models for 2 classes. The data set of 40 subjects was split into training (67%) and testing (33%)

sets. Evaluation metrics such as accuracy and area under the ROC curve AUC of the ML models were estimated and averaged over 1,000 bootstrapped repetitions.

**Results**. The highest mean prediction accuracy was obtained using DNN-A ( $80.41 \pm 3.89\%$ , AUC 79.17 ± 6.79).

**Conclusion**. The findings emphasize the potential of MEG data in diagnosing PD, providing a robust basis for future investigations.

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The aim of this study was to find out the changes in the levels of iron (Fe) and copper (Cu) related to organic and inorganic selenium (Se) compounds administration. Experiments were done on 4-6 weeks old BALB/c mice. Control mice were given tap water, whereas Se treated (0.2 mg Se/kg b.w.) mice – inorganic sodium selenite (Na2SeO3) or organic selenomethionine (C5H11NO2Se) in tap water for 8 weeks (License No. G2-80).

It was shown that exposure to selenomethionine and sodium selenite caused a statistically significant increase in Se level in mice blood in regard to control. Se concentration increased 6.53-fold (1374.66  $\mu$ g/L) and 3.26-fold (686.78  $\mu$ g/L), respectively. The exposure to selenomethionine decreased blood Cu and Fe concentrations by 38 % (0.61 mg/L, p<0.05) and 28 % (507.69 mg/L, p<0.05), respectively, as compared to control. Administration of sodium selenite increased blood Cu level by 15 % (1.13 mg/L, p<0.05) and did not induce statistically significant change in blood Fe level in mice as compared to control. The exposure to selenomethionine increased Se concentration in mice brain by 17.3-fold in comparison with Se concentration of control (0.061  $\mu$ g/g). Meanwhile, Se accumulation in the brain was 2.4-fold (0.15  $\mu$ g/g, p<0.05) higher as compared to control after sodium selenite administration. It was also determined an increase in brain Fe concentration by 24 % (33.75  $\mu$ g/g, p<0.05) and Cu concentration by 70 % (6.31  $\mu$ g/g, p<0.05) in regard to control after selenomethionine administration. The exposure to sodium selenite significantly increased Fe and Cu levels in the brain by 19 % (32.40  $\mu$ g/g) and 32 % (4.91  $\mu$ g/g), respectively, in regard to control.

In summary, exposure to selenomethionine for 8 weeks increased Se level 2-fold in blood and more than 7-fold in brain in comparison to Se level in mice exposed to sodium selenite. The consumption of different Se compounds disrupted both Cu and Fe homeostasis. Exposure to selenomethionine decreased the blood Cu and Fe concentrations, while exposure to sodium selenite, on the contrary, increased the blood Cu concentration in regard to control mice. Exposure to selenomethionine caused an increase in brain Cu level more than 2-fold as compared to exposure to sodium selenite. Meanwhile, the increase in Fe level in mice brain was quite similar after long-term selenomethionine or sodium selenite administration.

## Glioma classification and prognosis through lncRNA

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**Background:** 30% of all central nervous system tumors are Gliomas, which are generally considered as incurable. Thus, there is a desperate need for new findings that could help detect and classify gliomas, as well as estimate a more accurate prognosis for patients. Long non-coding RNA (lncRNA), far from their previous designation as "junk RNA", are emerging as important factors in various processes, both homeostatic and pathological.

Aim: To evaluate the utility of lncRNA in detection, classification, and prognosis of gliomas.

**Methods:** In-house primary tumor samples were obtained by collaboration with the "Kauno Klinikos" hospital department of neurosurgery and sequenced by direct RNA sequencing, using Nanopore technology. In-house data was integrated with the publicly available TCGA LGG and TCGA GBM datasets and run through the Seurat single-cell RNAseq R pipeline in order to cluster patients only utilizing lncRNA genes. LncRNA genes were extracted according to Genecode\_v42 lncRNA annotation.

**Results:** Graph-based clustering separated patients into 4 clusters. The "Glioblastoma" cluster had patients with the worst survival times, regardless of prior clinically based diagnosis. LncRNA-based "Glioblastoma" cluster had a higher hazard ratio than both "Glioblastoma" cluster obtained by utilizing all RNA and clinical glioblastoma diagnosis. 5 genes were designated as marker genes for the lncRNA based "Glioblastoma" cluster: MIR4435-2HG, ENSG00000275830, H19, BAALC-AS1 and NEAT1.

## The Influence of Structural Change on Filled Space and Filled Time Illusion Strength

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Time and space perceptual domains are intuitively understood as very different. However, evidences suggests that information from both domains shares the same structures in the human brain, and even some illusions are very similar. One such pair is filled space and filled time illusions, where a filled interval, whether it be time or space, appears longer than an empty one. The aim of this work is to present and compare some phenomenological aspects of these two illusions. The visual filled space illusory figure comprised two or three intervals, marked by vertical stripes. In the two-interval case, the first interval was filled with six vertical stripes, and in the three-interval configuration, the first and third intervals were filled. The filled interval was constant at 120 arc min. The filled time illusory stimulus was constructed out of auditory pure tone bursts. Analogous to the visual stimulus, there were two stimulus configurations in which the first and third time intervals of one second in duration were filled with six sound bursts. Twelve participants participated in the experiments. In the forced-choice procedure, they had to decide which interval appeared longer, the empty or the filled one. There were 51 trials for each stimulus configuration. The illusion strength was calculated as the difference between the empty and filled intervals when they were perceived as equal. Illusory effects were observed in all cases and for all participants. The average illusion strength was 28% for the two-interval visual stimulus and 22% for the three-interval version. For the auditory stimulus, the illusion strengths were 65% and 60%, respectively. For the given sample, both types of illusions acted in a similar manner, with an addition of second filled interval slightly reducing the illusory effect for both types of illusions. The results are consistent with the hypothesis that space and time information may share common processing principles in human brain.

## Pituitary adenoma development: does the TAS2R16 have an impact?

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Pituitary adenoma (PA) is a slow-growing benign tumor that usually arises from adenohypophyseal cells. PA is characterized by neurological symptoms such as headache, and changes in visual function, or endocrine symptoms such as infertility, decreased libido, and galactorrhea. The pathogenic mechanisms of PA are multifactorial and include interactions between mutated genes, impaired protein expression, and epigenetic alterations. The taste receptors of family 2 (TAS2R), which belong to the family of G-protein-coupled receptors, were first identified in the taste buds and function as peripheral taste receptors for bitter taste. TAS2Rs are not only found in the mouth and throat, but also in the intestine, brain, bladder, and lower and upper respiratory tract. TAS2Rs are also present and functionally active in carcinogenic cells. Given all to the consideration, we analyzed the TAS2R16 protein expression impact on the patients, who have a PA.

Aim: To evaluate the link between pituitary adenoma occurrence and TAS2R16 expression.

**Materials and methods:** The study included 20 patients with PA and 20 healthy individuals. Genotypes of the *TAS2R16* rs860170, rs978739, and rs1357949 single nucleotide polymorphisms (SNPs) were determined by using RT-PCR. TAS2R16 protein concentrations in blood serum were measured by the ELISA method. The obtained data were statistically evaluated using "IBM SPSS Statistics 29.0.1.0" software.

**Results:** TAS2R16 serum levels were measured in duplicates for 20 PA patients and 20 control subjects. Analysis showed elevated TAS2R16 serum levels in the PA group compared to control subjects (median (IQR): 0.147 (0.075) ng/ml vs. 0.116 (0.012) ng/ml, p<0.001). A comparison of TAS216 concentration between different genotypes of the *TAS2R16* rs860170, rs978739, and rs1357949 SNPs was also performed. PA patients with TT or CT genotype of *TAS2R16* rs860170 SNP have higher serum protein concentrations than healthy control individuals (p=0.031 and p=0.006, respectively). By analyzing the *TAS2R16* rs978739, we found that PA patients with TT or CT genotype have higher blood serum levels of TAS2R16 than healthy subjects (p=0.025 and p=0.019, respectively). PA patients with AA or AG genotype of *TAS2R16* SNP have higher serum protein concentrations than healthy control individuals (p=0.005 and p=0.007, respectively).

**Conclusion:** We found elevated TAS216 serum levels in the PA group compared to the control subjects (p<0.001).

## Influence of attention on the 40 Hz auditory steady-state response

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Auditory steady-state response (ASSR) has been increasingly researched as a potential biomarker of neuropsychiatric diseases such as schizophrenia and disorders of consciousness as well as for possible application in brain-computer interface technologies. Attention has been investigated as one of the factors that could impact the ASSR. However, studies of attention role in ASSR generation provide inconsistent results. Therefore, to further the progress of ASSR applications, it is important to continue research focused on understanding how attention can affect ASSR. The aim of the study was to evaluate the influence of attention on the generation of 40 Hz ASSR. Electroencephalography (EEG) was used to record ASSR elicited to 40 Hz click trains in twenty-three healthy subjects, during two experimental conditions: 1) concentration - counting presented stimuli while watching a video, 2) distraction - ignoring presented stimuli while watching a video. ASSR was evaluated using two measures: inter-trial phase coherence (ITPC) and evoked amplitude (avWT). Both measures were averaged across early (0-100 ms) and late (200-500 ms) latency ASSR. The averages of ITPC and avWT were compared between two attentional conditions. Significance of results was evaluated by applying paired samples t-test with 10 000 test repetitions. Study results revealed increased phase consistency (ITPC) with attention to presented stimuli for both early (p = 0.04) and late (p = 0.01) latency ASSR. In addition, evoked amplitude measurement showed increased response power during concentration condition for early (p = (0.02) and late (p = 0.01) latency ASSR. In conclusion, our findings show that attention to presented click stimulation affects the generation of 40 Hz ASSR.

## on MsrB1, SELENOS, Caspase-3 and GADD45 genes expressions in mouse liver and brain samples

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Selenomethionine (SeMet) is an organic form of selenium, an essential trace element that plays an important role in reproduction, thyroid hormone metabolism, DNA synthesis, immune response regulation. This study aimed to evaluate the effects of SeMet concentration on genes responsible for redox regulation (MsrB1), regulation of protein folding, immune and inflammatory processes (SELENOS) as well as genes related to apoptosis and cell cycle control (Caspase-3 and GADD45) in mice liver and brain. The study was performed on 4-6 week old BALB/c mice, which were divided into a control group that had free access to tap water, and groups that were given ad libitum tap water supplemented with different concentrations of selenomethionine (0.2 and 0.4 mg SeMet/kg of body weight) for the period of 8 weeks. After extracting mRNA from mouse liver and brain samples and performing complementary DNA synthesis, gene expression changes in the samples were determined by Real-time polymerase chain reaction. The obtained results showed that the expression of the Caspase-3 gene in both liver and brain samples was the highest among the control group, while the lowest expression was found among the mice that received 0.2 mg SeMet/kg. The expression of MsrB1 and SELENOS genes in the control groups of both samples is the lowest, while the expression of the mice exposed to 0.2 mg SeMet/kg is the highest. It was also found that with increasing SeMet concentration, GADD45 gene expression increased in liver samples and decreased in brain samples. According to the results, in mice treated with Selenomethionine, an increase in MsrB1 and SELENOS gene expression and a decrease in Caspase-3 gene expression in liver and brain samples, and an increase in GADD45 gene expression in liver samples lead to better cell survival associated with the crucial protection of these genes against inflammation, oxidative stress, DNA damage and apoptosis. However, as the concentration of SeMet increases, the decrease in GADD45 gene expression in brain samples may have a negative impact on cell functions, causing DNA damage.

## The effect of maternal high-fat diet and estrous cycle on microglia in female offspring retina

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**Aim:** Today's typical diet has high-fat content, and this leads to increasing obesity rates in human population. Many studies show that maternal high-fat diet (mHFD) causes systemic inflammation which can lead to neurodevelopmental disorders of the offspring. There is evidence that female estrous cycle stages different responses to inflammation. Although the retina, being a component of the central nervous system, is known to be affected by high-fat diets, there is a lack of studies investigating the impact of maternal high-fat diet on the retinas of offspring. We aimed to evaluate how different female offspring estrus cycle stages can affect inflammation response to mHFD in offspring retina.

**Methods:** We fed female C57Bl/6J mice with a control diet (CD, 10% fat) or high-fat diet (HFD, 60% fat) from weaning to lactation. The offspring were weaned to CD. The eyeballs of the offspring were collected, fixed with 4% PFA, cryoprotected, and sliced into 15  $\mu$ m thick sections using a cryotome. Microglia cells were labeled immunohistochemically using anti-Iba1 antibodies. The estrous cycle stages were determined by vaginal cytology in female offspring on the day of tissue collection (22 weeks old).

**Results:** We evaluated the tendency of increased Iba1-labeled (Iba1+) microglia number and area in the peripheral, but not central, retina of female offspring exposed to mHFD. We showed that Iba1+ microglia area in the periphery retina highly depends on the stage of the estrous cycle. Furthermore, mHFD altered Iba1+ microglia area in all stages of the estrous cycle in female offspring.

**Conclusions:** Our findings showed that mHFD diet had an estrous cycle stage-specific effect on activation of microglia in the periphery retina of female offspring

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## TNKS2 and TERF1 Gene Polymorphisms Associations with Pituitary Adenoma Recurrence

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**Introduction:** Pituitary adenomas (PAs) represent a diverse group of neoplastic lesions arising from the anterior lobe of the pituitary gland. While advances in surgical techniques and medical therapies have improved the prognosis for patients with pituitary adenomas, recurrence remains a difficult and poorly understood aspect of their management. In this work, the intricate relationship between genetic factors and the recurrence of pituitary adenomas is investigated, with a particular focus on polymorphisms in the TNKS2 and TERF1 genes. By exploring the molecular underpinnings of these genes and their potential association with the recurrence of pituitary adenomas, this research aims to identify new avenues for personalised treatment strategies and improved patient care.

**Materials and Methods:** The study involved 130 patients with pituitary adenoma and 320 healthy individuals. DNA samples from peripheral blood leukocytes were purified using the DNA salting-out method. Single nucleotide polymorphisms were performed by RT-PCR. The results were evaluated using the statistical analysis method "IBM SPSS Statistics 29.0 ".

#### **Results:**

- The TNKS2 rs10509637 AA genotype was associated with a 4.2-fold increased odds of PA recurrence.
- The TERF1 rs1545827 CT+TT genotypes were linked to a 3.5-fold decreased odds of PA without recurrence. While the TNKS2 rs10509637 AA genotype was associated with a 6.4-fold increased odds of PA without recurrence.

**Conclusion:** In conclusion, our study highlights the potential impact of specific genetic polymorphisms in the TNKS2 and TERF1 genes on the recurrence and development of pituitary adenomas. The TNKS2 rs10509637 AA genotype is associated with an increased odds of PA recurrence, while the TERF1 rs1545827 CT+TT genotypes appear to decrease the odds of PA recurrence. Furthermore, the TNKS2 rs10509637 AA genotype is associated with the occurrence of non-recurrent PAs. These findings highlight the intricate interplay of genetics in PA pathogenesis and recurrence and provide valuable insights for personalised treatment approaches and risk assessment.

## Effect of Phytocannabinoids on Rat Behavior

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Growing results from preclinical and early clinical trials suggest that modulation of the endocannabinoid system by endogenous or exogenous cannabinoids may be significant in the treatment of various diseases. The endocannabinoid system influences virtually all central nervous system processes: learning, memory, appetite, emotions, and more. Tetrahydrocannabinol (THC) is the main psychoactive substance found in cannabis that can lead to addiction. However, a whole series of other phytocannabinoids have been isolated, such as cannabinol (CBN), tetrahydrocannabivarin, (THCV) and Δ9-Tetrahydrocannabiorcol (THCC), which are not psychoactive but may have various therapeutic properties. The aim of this study was to investigate the effects of CBN, THCV and THCC on rat behavior. For this purpose, changes in the behavior were investigated 30 min after the administration of the test substances using classical tests for measuring behavioral changes in rats: i) the open field test had been used to assess changes in the animal's locomotor activity and anxiety-related behavior; ii) working memory was assessed by a novel object recognition test; (iii) the influence on social behavior was assessed by the number and duration of social interactions and number of ultrasonic vocalization with an unfamiliar social partner, and (iv) impact on the pain sensitivity was studied using a hot plate test Different doses of were used in the open field test in order to establish a safety profile of these cannabinoids. The doses that did not produce or produced mild sedative effect were used for the reminder of the experiment. Our results showed that even a very low dose of THCV (1 mg/kg) had a sedative effect on rats. Contrary, only very high doses of THCC and CBN reduced locomotor activity of animals. Neither of the tested substance affected short term memory, social behavior or pain sensitivity. However, administration of 5 mg/kg of THCV tended to increase a number of vocalizations expressing negative emotional state of rats. We conclude that administration of THCV may produce a sedative and aversive response in animals. CBN and THCC compounds demonstrated good safety profile and can be used for further studies. Chronic treatment regimen may be used to increase effectiveness of phytocannabinoids in treating pain-related illnesses.

## The effects of Cannabis sativa L. extract on antioxidant status in mice blood and organs

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Cannabis sativa L. extract is high in antioxidant components, including a wide range of terpenes, phenols, especially stilbenoids, and may play a role in reducing the risk of chronic diseases. Al exposure-related neurotoxicity is implicated to have a role in the etiology of neurodegenerative disorders. However, there is accumulating evidence that natural phenolic compounds with strong antioxidant activities could alleviate neuronal oxidative damage and inflammation and counteract metabolic disorders associated with these diseases. The present study aimed to elucidate possible protective effects of Cannabis sativa L. extract (CE) in alleviating the toxicity of Al on reduced glutathione (GSH) and malondialdehyde (MDA) concentrations as well as catalase (CAT) activity in brain and liver homogenates of mice organs. The experiments were done on 4-6-weeks old BALB/c mice weighing 20-25 g. 8 mice per group were assigned to 4 groups: control; 0.05 LD50 CE; 0.15 LD50 Al; 0.15 LD50Al+0.05 LD50 CE. CE were administered intragastrically to mice via a stomach tube for 21 days. The amount of intracellular antioxidant GSH was assessed based on GSH reaction with Ellman's reagent which produces a yellow complex. The amount of MDA (marker of lipid peroxidation) was estimated by measuring thiobarbituric acid reactive substances. The activity of CAT was determined by the hydrogen peroxide reaction with ammonium molybdate, which produces a complex that absorbs light at 410 nm. Results were expressed as the mean ± SEM. CE significantly decreased the concentration of reduced GSH in blood of mice affected with aluminum ions by 26.8% and stabilized the level of oxidative stress indicator to the level of these parameters in the control mice. CE significantly reduced MDA concentration in brain and liver of mice affected by aluminum ions, respectively, by 82.12% and 53.5%. CE significantly increased CAT in brain of control mice by 56.4% and mice affected by aluminum by 64.8% and, respectively, in liver by 34.5% and 72.4%. Cannabis sativa L. extract normalizes uncontrolled synthesis of reduced glutathione caused by aluminum ions, protects brain and liver lipids from peroxidation, and has a strong stimulating effect on the brain and liver antioxidant protection system.

## Long-term exposure to selenium excess disturbs trace elements homeostasis in mice brain

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Selenium (Se) as part of selenoproteins, is involved in the maintenance of brain redox homeostasis and the regulation of signal transduction pathways. Essential trace elements iron (Fe), zinc (Zn) and copper (Cu), termed "neurometals", also play an important role in the brain by participating in myelination, neuroprotection, modulation of synaptic activity and neuronal plasticity, and synthesis of neurotransmitters. Se interaction with essential trace elements has not been fully investigated. There is also little data on how an excess of Se affects the homeostasis of Fe, Zn and Cu. Excess of these trace elements, including Se, can produce reactive oxygen species and damage the brain. It is widely accepted that dyshomeostasis of these neurometals (excess as well as deficiency) is involved in the pathogenesis of various neurodegenerative diseases. Experiments were done on 4-6 week-old BALB/c mice. Control mice were given tap water, whereas Se treated mice - organic selenium compound selenomethionine (C5H11NO2Se, 0.4 mg of Se/kg body weight) in tap water for 8 weeks. Trace elements concentrations in mice brain and blood were determined by inductively coupled plasma mass spectrometry using NexION 300 D. (License No. G2-80). It was shown that after 8-week oral consumption of selenomethionine solution, Se concentration in mice blood increased 12.6-fold (from 210.51 to 2656.89 µg/L, p<0.05). Meanwhile Se concentration in brain increased 42.2-fold (2.57  $\mu$ g/g, p<0.05) in comparison with Se concentration value  $(0.061 \ \mu g/g, p < 0.05)$  of control. The exposure to selenomethionine caused a statistically significant decrease in Cu level in the blood by 40 % (0.59 mg/L) and increase in the brain by 69 % (6.27 mg/L) in regard to control. The administration of selenomethionine decreased Fe concentration in mice blood by 30 % (497.12 mg/L, p<0.05), while Fe concentration in the brain increased by 42 % (38.80  $\mu$ g/g, p<0.05) as compared to control (27.27  $\mu$ g/g). It was also determined an increase in brain Zn level (24.62  $\mu$ g/g) in regard to control (16.86  $\mu$ g/g) after selenomethionine administration. In conclusion, the exposure to selenomethionine for 8 weeks increases Se concentration in mice blood resulting in higher accumulation of Se in the brain, which respectively increases Cu, Fe and Zn levels. Meanwhile, the raised Se level in the blood is related to decreased Cu and Fe concentrations.

## Investigation of the Changes in Microglial Energy Metabolism upon Pro-inflammatory Stimulation with Amyloid Protein S100A9 and LPS

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Microglia, the resident macrophages of the central nervous system, play a crucial role in the pathogenesis of most common neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Under pathology, microglia activate and exhibit a chronic pro-inflammatory phenotype, leading to neuronal damage. Microglial activation leads to metabolic reprogramming, which acts as a key driver of the immune response. Neuroinflammation is a complex process and several factors contribute to microglial activation. Inflammation may be triggered by the accumulation of pathological proteins in neurodegenerative diseases. Recently, the expression of the pro-inflammatory S100A9 protein was shown to be dramatically upregulated in AD and PD. Another hypothesis postulates that endotoxins (LPS) from gut bacteria contribute to the pathogenesis of neurodegenerative disorders via neuroinflammation. LPS and S100A9 can directly activate microglia; however, the microglial metabolic changes during the inflammatory response are not fully understood. In this study, using high-resolution respirometry, we investigated metabolic changes in the microglial BV-2 cell line in response to LPS and pre-aggregated recombinant S100A9 protein. We found that both LPS and S100A9 significantly reduced the oxidation of mitochondrial complex substrates I and II, leading to reduced ADP phosphorylation levels. LPS and S100A9 did not alter mitochondrial inner membrane permeability or uncouple oxidative phosphorylation. Overall, our data suggest that LPS and S100A9 induced microglial metabolism changes could contribute to neurodegenerative processes through reduced oxidative phosphorylation.

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## What is the utility of the tasks in immersive virtual reality for executive functions and emotional state improvement in stroke patients?

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**Aim.** The aim of this pilot study was to test the feasibility and effectiveness of short-term visual memory tasks in immersive virtual reality on executive function and emotional state in stroke patients.

**Methods.** Twenty-seven stroke survivors, who were inpatients in Abromiškės rehabilitation center, were randomly allocated to one of the two groups (Immersive virtual reality – iVR, and Control). All participants underwent pre-assessment and post-assessment. Furthermore, all participants participated in a conventional rehabilitation program. Additionally, the participants in the iVR group underwent 10 sessions of short-term visual memory training in the iVR environment. The time length between pre-assessment and post-assessment was two weeks. Cognitive functions were tested by the Trail Making Test A and B. Emotional state was evaluated by Patient Health Questionnaire-9 and General Anxiety Disorder-7. The final data analysis included 20 participants (mean age –  $62,15\pm7,8$ ) because 7 did not complete the post-assessment.

**Results.** Our results revealed significant differences just comparing the results within iVR group patients. Our results reveal that the patients in the iVR group (N=13) improved their visual search abilities (M1=71.54, SD1=58.75, M2=54.08, SD2=36.75, p=.041) with the small effect size (Hedges'g=0.35), also improved their task-switching abilities (M1=125, SD1=89.82, M2=102.46, SD2=69.36, p=.025) with the small effect size (Hedges'g=0.27). Furthermore, patients in the iVR group lower their depression rate (M1=3.92, SD1=3.06, M2=1.92, SD2=2.39, p=.021) with the large effect size (Hedges'g=0.77), and anxiety rate M1=4, SD1=2.86, M2=1.67, SD2=2.84, p=.005) with the large effect size (Hedges'g=0.79). Meanwhile, results within the control group did not show any significant differences. Discussion. In accordance with the existing body of research, our results indicate that iVR is feasible and safe to use in rehabilitation settings for stroke patients. The presented evidence suggests that innovative tools can boost conventional rehabilitation by optimizing the neuroplasticity and as a result enhance not just near-transfer but also the far-transfer effect.

**Conclusions.** Short-term visual memory tasks in immersive virtual reality are feasible and effective for the improvement of visual search and task-switching abilities. Furthermore, it can enhance the far-transfer effect which results in an improved emotional state.

## **Psychology Students' Attitudes towards Artificial Intelligence in Mental Health**

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**Background and aim.** While artificial intelligence (AI) and its application in medicine are developing rapidly (Liu et al., 2018), its integration into mental health care is nascent (Fiske et al., 2017). The aim of this study is to evaluate the attitudes of future psychologists towards AI systems and their application in mental health care.

**Methods.** The study included 34 psychology students (mean= $23,1 \pm 5$ ; 86% females) with 18 assigned to the Wysa group and 17 to the second group. The first group used Wysa, a mobile app offering emotional support through AI-driven chat conversations. The second group watched a recorded presentation on AI in mental health. Attitudes towards AI were evaluated pre- and post- interventions in both groups using The General Attitudes towards Artificial Intelligence Scale and Oh et al. (2019) questionnaire. During interventions, automated facial expression analysis software "FaceReader" was used to assess participants' emotions.

**Results.** Significant differences in emotional states emerged between the groups, with the Wysa group showing significantly higher levels of the emotions "angry", "contempt", "disgusted", "happy" and "scared" (Mann-Whitney test p=0.02; 0.03; <0.001; <0.001; 0.09, correspondingly) while the presentation group demonstrated a significantly higher prevalence of the "neutral" state (Mann-Whitney test p<0.00). Moreover, we investigated the differences in the attitudes between the presentation and Wysa groups. Before the intervention, the scores of "Perception of AI technologies in psychology" did not differ significantly (Mann-Whitney test p=0.252). Post-intervention, participants of the presentation group showed a higher positive attitude compared to the Wysa group (Mann-Whitney test p=0.010). Next, we analyzed the changes in the attitudes within the two groups. After the intervention, participants in the presentation group reported higher scores on the "Perception of AI technologies of using AI in psychology" and "Positive attitudes towards AI" (Wilcoxon signed rank test p=0.001; 0.013; 0.017, correspondingly). Participants of the Wysa group did not show significant changes in the attitudes on AI technologies.

**Conclusion**. The presentation intervention elicited significant positive changes in attitudes towards AI in psychology, whereas the Wysa intervention did not yield statistically significant alterations in attitudes among participants.

### aSyn aggregates induce microgliadependent neurotoxicity

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Parkinson's disease (PD) is neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in substantia nigra pars compacta resulting in movement dysfunction. The main histological hallmark of PD is the presence of intracellular fibrillary aggregates, so-called Lewy bodies (LB). The formation of LB is considered the main cause of neuronal death in PD, but LB toxicity is currently under debate. Emerging evidence indicates that soluble aSyn oligomers are also toxic to neuronal cells. In addition, aSyn oligomers elevate in the extracellular space (cerebrospinal fluid) and are capable of spreading cell-to-cell. Importantly, extracellular aSyn can affect both glial and neuronal cells. However, the interaction of brain cells under pathological conditions requires further investigation. Using rat neuronal-glial co-cultures, we found that pre-aggregated recombinant aSyn caused a gradual loss of neurons. Elimination of microglial cells abolished aSyn neurotoxicity, suggesting that microglial cells mediate neuronal loss. We also found that aSyn-induced neuronal loss was accompanied by the production of pro-inflammatory factors, such as nitric oxide and TNF- $\alpha$ . Overall, our results show that extracellular pre-aggregated aSyn induces microglia-dependent neurotoxicity in neuronal-glial co-cultures.

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## The influence of cerebrospinal fluid on neural stem cells' fate

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We have already seen that human neural stem cells (hNSCs) show promising outcomes when used for cell therapy in neurological disorders treatment, however, several issues should be further addressed even on the preclinical level. Neurogenesis is still not enough explored, thus, more research is needed to investigate the limitations and effectiveness of the therapy and to investigate which laboratory conditions could mimic the physiological environment of the brain. It has been reported previously that the cerebrospinal fluid (CSF), as a natural component of the brain niche, plays a vital role not only in brain development, but also in NSCs' survival, proliferation, and differentiation processes. However, its exact role in adult neurogenesis is much less clear. There is a minimal number of data regarding its influence on NSCs. Therefore, in our study, we preincubated human NSC line with the human CSF leftovers from healthy donors (in whom the reason for collecting CSF was the suspicion of neurological disease that has not been confirmed) to obtain closer to the physiological brain environment and to assess NSCs' fate and their therapeutic abilities in vitro and ex vivo. We observed significant differences in the secretory potential of CSF-treated NSCs, and, moreover, their elevated neuroprotective potential after co-culture with ischemically damaged by oxygen-glucose deprivation (OGD) organotypic rat hippocampal slices culture (OHC) in comparison to the cells cultured in the standard conditions. This study exposed the critical importance of nutritional supplementation regarding NSC culture maintenance and therapeutic properties and brings hope for understanding the mechanisms underlying brain function and disease, which may ultimately lead to the development of new therapeutic interventions for neurological disorders. keywords: neural stem cells, ischemic stroke, cerebrospinal fluid, neuroprotection, cell therapy

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## The Future of Alzheimer's Diagnosis: Integrating Novel Biomarkers and Al Insights

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Alzheimer's disease (AD) is a complex neurodegenerative disease and the most common type of dementia. The identification of AD biomarkers is an increasingly expanded area of research. The development of new technologies enables the determination of various AD biomarkers, including pathogenic proteins, markers of synaptic dysfunction, and markers in the blood. Despite the widespread use of cerebrospinal fluid (CSF) biomarkers and PET imaging, there is a growing trend to include plasma biomarkers such as Aβ40, Aβ42, P-Tau and pro-inflammatory cytokines in clinical trials. Blood-based biomarkers, particularly miRNAs, due to their demonstrated high sensitivity and specificity, could significantly reduce the cost of AD diagnosis, making low-cost screening available to a broader population. Improved visualization methods for retinal studies also hold potential for diagnosing AD. The integration of new AD biomarkers is expected to accelerate clinical development and increase the accuracy and specificity of diagnosis. Artificial intelligence (AI)-based approaches have demonstrated promise in uncovering novel biomarkers for dementia, yet they come with challenges. Addressing these hurdles could result in the discovery of clinically valuable biomarkers that are accurate, applicable across diverse cases, unbiased, and suitable for integration into clinical practice.

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### Vision in Bengal owl is slow

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To interact with visual environment animals need to accurately identify and locate moving objects. Precise tracking of fast-moving objects, both in space and time, is especially important for flying animals. As nocturnal predators, owls are believed to possess a visual system, which is adapted to function well in dim light conditions, resulting in decreased spatial resolution in exchange for better sensitivity. Though some studies have investigated spatial resolution in owls, there is very little knowledge on their ability to resolve visual detail in time. Owl temporal resolution has never been tested with behavioural methods before. Using a two-choice discrimination task a Bengal owl (Bubo bengalensis) was trained to discriminate a flickering light from a constant light. Stimuli were generated by 2 cm diameter white LED lamps. To distribute light evenly and to achieve different stimuli intensities, diffusion and neutral density filters were used. After the owl has successfully learned the task, different flicker frequencies were presented in ascending order, starting from 10 Hz, until the owl could no longer discriminate the flickering from the constant light. The procedure was repeated three times at each different light condition and the critical flicker fusion frequency (CFF) - the highest flicker fusion frequency at any light intensity - was determined. The frequency of flickering light, when the owl could no longer discriminate it from the constant light, was  $43.33 \pm 0.47$  Hz (mean  $\pm$  s.d.; n = 3 sessions), measured at 30000 cd/ m2 stimulus luminance, and was determined as CFF of the Bengal owl. Flicker frequencies up to  $33.66 \pm 0.82$  Hz (n = 3 sessions) were discriminated by the owl at 55500 cd/m2 stimulus luminance and  $17.00 \pm 0.01$  Hz (n = 3 sessions) at 3200 cd/m2. These results place the Bengal owl at the very low end of the temporal resolution spectrum of birds. The low CFF of Bengal owl reflects contrasting differences when compared to some diurnal birds of prey, which are known to demonstrate high capabilities of rapid vision aiding in catching prey, avoiding predators and navigating their environments. Therefore, our results imply that rapid vision is not a trait that allows Bengal owl to be an effective, precise and secrete hunter under low light conditions. Whether low temporal resolution is typical in different owl species remains to be tested.

## The Effect of Hyperoside Solution on Malondialdehyde (MDA) in Mice Brain

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**Background**. Hyperoside is one of the active components mostly found in H. perforatum L. Hyperoside has been associated with numerous biological activities that affect physiological processes of the organism. For example it is known to have antioxidant, anti-inflamatory and neuroprotective effects. Due to its antioxidant properties hyperoside is hypothesized to alleviate oxidative stress [1]. It is a condition when the balance between the production of free radicals in the body is disturbed. This condition can affect the damage of various organ systems, cells and DNA [2]. Malondialdehyde (MDA) is a traditional biomarker of oxidative stress, elevated MDA levels can indicate increased oxidative damage [3].

**Aim**. The aim of this study was to determine the effect of hyperoside solution to oxidative stress on MDA concentrations in the mice brain after AlCl3 exposure.

**Methods.** Experiments were performed on 4 – 6 weeks old BALB/c mice. Mice were randomly apportioned to four groups with 8 mice per group – control, AlCl3, hyperoside and a group that received both aluminum (AlCl3) and hyperoside after 20 minutes. The mice were intragastrically administrated hyperoside solution for 21 days. MDA concentrations in the brain were determined using spectrophotometry at 535 and 520 nm.

**Results.** Results showed that aluminum increased MDA concentrations in the brain of mice by 13 % compared to the control group ( $\geq 0,05$ ). Hyperoside increased MDA concentration by 5% in comparison with control mice too ( $\geq 0,05$ ). However, administration of hyperoside statistically significantly decreased MDA concentration in aluminium-treated mice brain by 54% compared to the aluminium-treated group ( $\leq 0,05$ ).

**Conclusion.** The ability of hyperoside to reduce elevated MDA concentration in the brain of mice after exposure of aluminum suggests that hyperoside has antioxidant properties. However, it would be appropriate to study the influence on the concentration of other oxidative stress markers as well, such as glutathione, catalase, etc.

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## The Role of KDR Genetic Variants in Females with Multiple Sclerosis

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**Introduction.** Multiple sclerosis (MS) is a chronic disease affecting the central nervous system [1], with its development potentially shaped by substantial genetic factors [2]. Considering the potential involvement of KDR in the pathogenesis of MS, our study concentrates on exploring gene polymorphisms [3]. The study aims to examine the connections between KDR and MS specifically among females.

**Aim.** The aim of this study was to determine the association between KDR genetics variants and MS among females.

**Methods.** Study enrolled 106 patients with MS, and 116 healthy controls. DNA was extracted from peripheral blood leukocytes using DNA salting-out method. Genotyping was carried out using real-time polymerase chain reaction (RT-PCR) method. Statistical analysis was performed with "SPSS version 27.0".

**Results**. The study did not find statistically significant differences in the occurrence of genotypes and alleles for KDR rs2305948 in relation to multiple sclerosis among females. Nevertheless, the binary logistic regression analysis demonstrated that the KDR rs2305948 CT genotype is associated with a two-fold increase in the odds of developing MS (OR = 2.058; CI: 1.018–4.160; p = 0.045).

**Conclusions.** We found associations between KDR rs2305948 and MS development among women.

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## Maintaining the neural phenotype of ADMSCs after changing culture conditions

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Mesenchymal stem/stromal cells derived from adipose tissue (ADSCs) are multipotent cells that meet the criteria established by the International Society for Cellular Therapy (ISCT). ADSCs are characterized by the fibroblast-like morphology, mesodermal differentiation capacity, clonogenic potential and expression of surface markers: CD105, CD73 or CD90 with the lack of express of CD34 and CD14. Because of such a general criteria, many people classify MSCs as a type of fibroblast or fibroblasts in a specific stage of development and deny their classification as a stem cells. Therefore, the aim of our study was to verified the phenotype differences, proliferative and clonogenic potential and the level of stem related transcriptional factors of that two populations derived from the same individuals. As the first step, we screened several potential reference genes for these two different populations in order to select the ideal reference for the next steps of this study. Finally, we are planning to compare the answer - differentiation potential of abovementioned populations toward neural phenotype – in response to neuromorphogenes (N21) and after their withdrawal.

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## Cognitive training with the use of the computer games based on spatialnumerical vs spatial-non-numerical association in children with dyscalculia risk

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The beneficial effect of cognitive training using computer games on the level of mathematical skills has been widely proven, and it is used in education and therapy, e.g. in dyscalculia. However, some methodological limitations (no control game or no passive and healthy control groups) make it difficult to compare the results obtained in studies and to make clear conclusions. Sixty eight children (8-11 years old) with the risk of dyscalculia participated in the study and they were divided into 3 groups: 21 children undergoing cognitive training with the computer game Kalkulilo (based on numerical-spatial relationship); 23 playing with the control game (using non-numeric symbols); and 19 with no training. They performed two (pre- and post-test) computer tasks measuring the level of basic numerical skills: number comparison, Numerical Stroop, numerosity estimation, number line estimation (0-100 and 0-1000 range). The cognitive training lasted 5 hours and was divided into 8-10 sessions of 30-45 min. each. The results showed the shorter reaction times in Numerical Stroop and the greater precision of 0-1000 number line estimation after the training with the Kalkulilo game. Although the effect of both Kalkulilo and the control game showed in individual participants in the results of several tasks, there were no clear differences at the group level. The probably reason is the great heterogeneity of the sample, observed even at the pre-test (children differ in the level of dominant deficit symptoms, which reflects the existence of several types of dyscalculia) and at the post-test (individual participants showed improvement, but in the level of various skills - e.g. only in numerosity estimation or in number line estimation). These results are consistent with the discussion concerning, on the one hand, difficulties in diagnosing dyscalculia, as well as developing and demonstrating the effectiveness of cognitive training and the transfer of trained to non-trained skills. This study was supported by the National Science Centre, SONATA BIS grant, number 2017/26/E/ HS6/00033.

Key words: mathematical cognition, computer games, mental number line, dyscalculia

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### A low-cost, open-source system for monitoring locomotor activity of rats

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Long-term locomotor home-cage activity monitoring can provide significant insight into general well-being and circadian patterns in animal models. Compared to other behavioral tests, such an approach allows the creation of a low-stress environment, which might reveal more naturalistic patterns in animals. A common barrier to monitoring home-cage animal activity during experiments is the cost of such systems. Here, we present a low-cost, scalable and adaptable solution for monitoring rat locomotor activity, as well as differences in animals after pharmacological intervention that were revealed using this equipment. The recording system includes infrared movement sensors, an Arduino microcontroller, and a computer that receives data. Animals are single-housed, and the sensor is placed above the cage in a 3D-printed chassis. When the recording is started, data from sensors is collected by the microcontroller and sent to the computer, which records it in a file. Two versions of the system were created, differing only in software. The original version, used in the experiment described below, records periods where the sensor turns on and off, and marks time in the computer system's timestamps, which allows one second accuracy. Writing to file is done via the Bonsai reactive programming interface. A new version utilizes the internal clock of the microcontroller, which lets us achieve an accuracy of one millisecond, and allows the use of a console software, such as Realterm, to record data to a file. The resulting file can be analyzed using Python or other software of choice. The analysis includes integrating movement durations over chosen periods. Without the computer, the system for 14 cages costs around 160 euros, which is significantly lower than other commercially available options. During our experiments, the system revealed activity changes after cannabinol (CBN) injections - namely increased activity two hours after injection, and decreased movement during the night period, when rats are naturally more active. Importantly, animal movement was constrained as they were also wearing electrophysiological recording equipment, so the total time the animal spent moving does not exceed two hours per day and is usually under one hour. In other types of experiments, where the animals move more freely, this duration should be expected to be longer.

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Parkinson's disease (PD) is one of the most common neurodegeneration disorders worldwide, related to the gradual degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta region of the brain in the presence of neuronal intracellular  $\alpha$ -synuclein ( $\alpha$ Syn) aggregates known as Lewy bodies (LB). Numerous scientific studies suggest that  $\alpha$ Syn may be associated with viral infections contributing to neurodegeneration. Still, there is a lack of evidence about the link between  $\alpha$ Syn, viral infection, and their synergistic effect on inflammatory responses in the brain.

The aim of this study was to investigate the synergistic effect of viral RNA-mimetics – loxoribine (LOX) and polyinosinic–polycytidylic acid (Poly (I:C) with amyloidogenic protein aSyn on primary rat neuronal-glial co-cultures (CGC).

In this study, we used primary neuronal-glial co-cultures (CGC) prepared from the cerebellum of 5–7-day-old Wistar rats pups (both genders). For experiments, culture cells were pre-incubated for 1 hour with RNA-mimetic LOX (1 $\mu$ g/ml) and Poly (I:C) (100 ng/ml). Then  $\alpha$ Syn (10-50nM), pre-aggregated for 6 h, was added, and cultures were incubated for 72 h. The viability and number of neuronal, microglia, and astrocyte cells were assessed by fluorescence microscopy.

We showed that LOX and Poly (I:C) incubated with or without 6 h pre-aggregated  $\alpha$ Syn did not affect CGC cell culture viability, which remained above 95 %. It was also found that various concentrations of  $\alpha$ Syn did not affect neurons. In contrast, 6 h pre-aggregated  $\alpha$ Syn (25nM – 50nM) incubation with viral Poly (I:C) and  $\alpha$ Syn (50nM) incubation with LOX decreased neuronal cell number and increased or tended to increase the proliferation of microglia by both – LOX and Poly(I:C) compared to the control group after 72 h incubation.

These results indicate a potential synergistic effect between  $\alpha$ Syn and viral RNA-mimetics in neuronal-glial co-cultures.

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## Optic Neuritis: the Influence of Relative Leukocyte Telomere Length and TERF1 (rs10107605) Gene Polymorphism

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**Introduction:** Optic neuritis (ON) involves primary optic nerve inflammation (1). It can be related to various systemic autoimmune disorders (2). TERF1 (Telomeric repeat binding factor 1), a major telomeric DNA-binding protein of the shelterin complex, plays a critical role in telomere protection, sister telomere resolution, and telomere length alteration (3, 4). Telomere length shortening is the result of the combined effects of oxidative stress, inflammation, and repetitive cell replication on telomeres, thus linking telomere length, chronological aging, and associated diseases (5).

**Methods:** A case-control study was performed with 210 subjects divided into two groups: 70 patients with ON and 140 healthy subjects. DNA samples from peripheral blood leukocytes were purified by DNA salting-out method. Single nucleotide polymorphism (rs10107605) was determined using real-time polymerase chain reaction (RT-PCR), while relative leukocyte telomere length was determined by qPCR. Results were analyzed using the "IBM SPSS Statistics 29.0" statistical analysis method.

**Results:** Subjects were categorized into two groups based on either long or short telomere length. Among those with short telomeres, there was no statistically significant difference in telomere length (mean (SD): 0.331 (0.121) vs. 0.284 (0.153), p=0.082). For subjects with long telomeres, there was a significant difference in telomere length (mean (SD): 1.380 (0.992) vs. 0.742 (0.137), p<0.001) indicating that the ON group had significantly longer telomeres than the control group subjects. When examining the distribution of TERF1 rs10107605 genotypes among patients with ON and control group subjects with short telomeres, no statistically significant results were observed. However, among those with long telomeres, the frequencies of the TERF1 rs10107605 genotypes (AA, AC, and CC) statistically significantly differ between the ON and control groups (78.4%, 21.6%, and 0% vs. 80.4%, 8.9%, and 10.7%, respectively, p=0.038).

**Conclusions:** Our study found significant differences in TERF1 rs10107605 genotypes between the ON and control groups among individuals with long telomeres (p=0.038). Patients with ON also exhibited notably longer telomeres compared to control group subjects (p<0.001). These results suggest a potential role of the TERF1 gene in ON, possibly through its impact on telomere length regulation. Further research is needed to explore this intriguing association and its implications for ON.

## The effect of the anesthetic propofol on connexins of cardiac and nervous system

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Propofol is widely used general anesthetic, which causes a rapid induction of anesthesia and rapid recovery after it. Propofol can also cause several side effects such as high incidence of blood pressure reduction. Scientific literature provides limited and often controversial information about the possible side effects of propofol on patients undergoing surgery. For example, propofol is believed to have a protective effect against ventricular arrhythmias during myocardial ischemia, but it was also shown to dramatically reduce the protective effect of remote ischemic preconditioning and induce arrhythmias. There is evidence that the mechanism of propofol action is related with GABA receptors; however, the side effects of propofol may manifest itself through its action on other targets. Indeed, several studies have showed that propofol may regulate cell coupling through gap junction (GJ) channels formed of connexin-43 (Cx43) in astrocytes possibly through protein phosphorylation by protein kinase C (PKC). Cx43 together with Cx40 and Cx45 are also expressed in cardiovascular system, and the ability of GJs to pass electrical signals ensure propagation of action potential in cardiac tissue.

The aim of this study was to compare the effect of propofol concentrations on GJs formed by Cx40, Cx43 and Cx45, which were expressed exogenously in human cervix epithelial adenocarcinoma cells (HeLa). The junctional conductance was measured using double wholecell patch clamp method. Our results showed that Cx40 and Cx43 channels exhibit similar sensitivity to various concentrations of propofol, while Cx45 channels are only sensitive to higher propofol concentrations as compared to Cx40 and Cx43.

The kinase inhibitor GF109203X was used to better understand the mechanism of propofol action on GJs. The low concentrations of GF109203X specifically inhibit PKC while higher concentrations inhibit both PKC and protein kinase A (PKA). Our data showed that low (40 nM) concentration of GF109203X significantly reduced inhibition of Cx43 channels conductance by propofol, indicating that propofol regulation of Cx43 channels is PKC-dependent. In contrast, GF109203X had no effect on inhibition of Cx40 channels by propofol, indicating that neither PKC nor PKA is involved in regulation of these channels by the anesthetic. This initial data suggest that propofol can have a prominent effect on GJs, which is connexin-specific.

## Transmission of Action Potentials through Internodal Cells of Nitellopsis Obtusa: Investigation of the Effect of Glutamate

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Action potentials (APs) are inherent in both animals and plants and play a pivotal role in adaptive plant responses influencing changes in respiration, photosynthesis, and osmotic pressure. Glutamate (Glu), a key neurotransmitter, acts as a signalling molecule in plants, functioning both in the ambient environment and internally. For instance, an increase in external Glu levels increases the excitability of plant cells, resulting in APs with greater amplitude. The impact of these changes on AP transmission throughout the entire plant body is uncertain. Characean macroalgae offer a reliable model for studying cell-to-cell electrical signalling. The tandem of two cells in a thallus (internodal cell-multicellular node-internodal cell) offers a concise system for studying AP propagation in plants. This study focuses on the electrical signalling between tandem cells of <em>Nitellopsis obtusa</em> (Characeaen) and aims to investigate the effect of external Glu on transmitting electrical signals locally. For this, intracellular glass electrodes were impaled in each internodal cell, and the two-electrode current-clamp technique was applied in each cell. The membrane potential in each cell was recorded, and three APs were elicited by increasing the current until the excitation threshold was reached, the process repeated every 5 minutes. This was iterated bidirectionally, signifying the initiation of APs in the apical cell and their transmission to the basal cell, and vice versa. This bidirectional process yields crucial insights into dynamic cellular communication within plants. Results indicate that in standard conditions AP propagates to the apical direction at a higher velocity than the basal one. However, the percentage of transmitted APs was low (46 %), and the signalling in the adjacent cell was predominantly driven by receptor potentials. Exposing the tandem to 1 mM Glu after control measurements did not reveal a clear Glu effect: transmission between neighbouring cells was inconsistent, occurring at times and absent at others. More research is needed to fully explore Glu's impact on tandem cell AP transmission and its effects on electrophysiological parameters.

## Antioxidant Effect of Plant Extracts in Mice Brain

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The brain is the tissue most vulnerable to oxidative damage because of its high rate of oxidative metabolic activity, intensive production of reactive oxygen metabolites, relatively low antioxidant capacity, low repair mechanism activity, nonreplicating nature of its neuronal cells, and the high membrane surface to cytoplasm ratio. The high concentrations of polyunsaturated fatty acids in the membrane lipids of the brain are the source for the decomposition reactions termed "lipid peroxidation," in which a single initiating free radical can precipitate the destruction of adjacent molecules. To contend with the continuous exposure to reactive oxygen species, the living cell has developed several lines of defense. These include preventive mechanisms against oxidative damage, repair mechanisms, and an antioxidant defense system. It is known, that one of the major low-molecular weight antioxidants in the brain is tripeptide glutathione (GSH). Antioxidants are compounds found in food sources. They play an instrumental role in protecting enzymes, fats, and vitamins in the body. These natural substances help to delay or prevent certain types of damage to the cell. Brightly colored fruits and vegetables are a great source of antioxidants. So, our study was performed to evaluate the antioxidant effects of red orange, grape seed and blackcurrant extracts on the brain of laboratory mice. Experiments were performed on outbred white laboratory mice by changing drinking water with red orange, grape seed and blackcurrant extract solutions. Exposure time was 21 days. Lipid peroxidation level was estimated spectrophotometrically by measuring the concentration of MDA produced by reaction with TBA at 535 nm and 520 nm light wavelengths. The concentration of GSH was measured spectrophotometrically by reaction with DTNB to give compound TNB, which absorbs light wavelength at 412 nm. Experiments showed that GSH concentration in mice brain significantly increased in all experimental groups (by 34% (blackcurrant extract), 42% (grape seed extract) and 184% (red orange extract)) as compared to the control mice group. The amount of MDA in mice brain significantly decreased by 54% in blackcurrant extract group, 44% in grape seed extract group and 37% in red orange extract group as compared to control. Our studies showed that blackcurrant, grape seed and red orange extracts protected lipids from oxidation and increased antioxidant GSH concentration in mice brain.

# The search for relationships between brain oscillations and fluctuations of sex steroid hormones

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Fluctuations in sex steroid levels during the menstrual cycle and the use of hormonal contraceptives have been linked to changes in female cognitive function and emotions. Such variations might be mediated by overall brain activity and excitability. We aimed to investigate the impact of females' hormonal status on resting-state EEG (rsEEG) parameters, including periodic (individual alpha frequency, alpha power) and aperiodic (1/f slope) features. rsEEG was recorded in healthy females ( $26.4\pm4.6$  years), who were naturally cycling in the early follicular (n=33) or mid-luteal phases (n=35), or who used either oral contraceptives (n=35) or hormonal intrauterine devices (n=28). Salivary concentrations of estradiol, progesterone, and testosterone were measured. Contrary to previous findings, the study did not reveal significant differences in rsEEG parameters between the groups or significant relationships with hormonal levels. Age emerged as a covariate negatively related to the median 1/f slope. These findings suggest that the resting state excitatory/inhibitory balance in the brain does not differ between the groups of females under investigation. Therefore, differences in task-related EEG or behavioral outcomes obtained in studies involving these groups are unlikely to be attributed to baseline electrical brain activity differences.

### Analysis of ncRNA Modifications' Affecting Proteins in Glioma Tumors

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Epigenetic modifications of non-coding RNAs (ncRNAs) have received increasing attention in recent years. Various new modifications are discovered, as well as the proteins that write, read and erase them, called "writers" (enzymes that deposit modifications), "erasers" (enzymes that remove modifications) and "readers" (proteins that recognize and bind epigenetic modifications). Epigenetic regulation of genes involved in cell proliferation, survival, and differentiation is believed to be involved, at least in part, in the initiation, development, and malignancy of various types of tumors. Gliomas - quite common malignant brain tumors with a poor prognosis - no exception. A number of publications have discussed the role of N6-methyladenosine (m6A) and 5-methylcytosine (m5C) modifications and their regulatory proteins in the aforementioned brain tumors. However, data on various other modifications such as pseudouridine ( $\Psi$ ), N7-methylguanosine (m7G), 5-hydroxymethylcytosine (hm5C), 5-N1-methyladenosine (m1A) and others, as well as their regulatory proteins in gliomas are rarely found. Since the relationship between these modifications and their regulatory proteins has been at least partially described in other types of tumors, the aim of this study was to investigate the differences in the expression of genes encoding various "writers", "readers" and "erasers", influencing ncRNA modifications in tumor samples from glioma patients of different malignancy grades. Several ncRNA modifications were selected for analysis: pseudouridine ( $\Psi$ ), N7-methylguanosine (m7G), 5-hydroxymethylcytosine (hm5C), 5-N1-methyladenosine (m1A). The expression of twelve genes encoding proteins regulating these modifications - FTO, ALKBH3, ALKBH5, BUD23, METTL1, DKC1, TET1, TET2, TET3, TRMT6, YTHDF1, YTHDC1 - as well as two reference genes -GAPDH and BActin - was evaluated using the qRT-PCR method. The study group consisted of 54 patients diagnosed with various malignancy grade astrocytoma. Gene expression was associated with tumor malignancy grade in most of the analyzed cases, and overall survival was significantly longer in astrocytoma patients with higher gene expression than the median of the analyzed genes.

## Sex Effect on Spontaneous and Evoked Brain Activity in Mice

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Electrical oscillations within neural networks reflect brain function. These oscillations have been associated with various brain functions and are altered in several psychiatric disorders. The assessment of the oscillations can be obtained from the brain's spontaneous activity or by inducing oscillations with sensory stimulation at a particular frequency, testing how the brain synchronises with the stimulus (Brenner et al., 2009). Oscillations are implicated by physiological factors such as emotional state, motor activity, or the estrus cycle (Jasinskyte et al., 2023; Li et al., 2020; Wang et al., 2019). Some evidence suggest that males and females exhibit different spontaneous (Sigalas et al., 2017) and evoked (Melynyte et al., 2018) oscillations, whereas other studies indicate no difference (Larsen et al., 2018; Tang et al., 2023). The aim of this study was to use both male and female mice to identify sex-related differences in brain oscillations. A total of 27 female and 34 male wild type C57BL/6 (PN70-PN91) mice were used in this study. Electrodes were implanted in the primary auditory cortex (A1) for electrocorticogram (ECoG) registration. Auditory steady-state responses (ASSRs) were induced by presenting 2 ms white noise stimuli (clicks) at 10, 20, 40 and 80 Hz for duration of 1 second, at 70 dB, with 1 second intervals between each stimulation. Signal timefrequency analysis was conducted with the Morlet wavelet transformation. The parameters of ASSR (power and phase-locking index (PLI)) were calculated. The results indicate that there are no sex differences in the spontaneous brain activity across all frequency ranges in mice. Moreover, the evoked brain activity (ASSRs) are similar in female and male mice at all frequency stimulations. In conclusion, no significant differences were observed in either the spontaneous or evoked brain activity between female and male mice.

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## Generation of SH-SY5Y cell line stably expressing EGFP and mRUBY2 reporter genes using CRISPR-mediated HDR

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Genome editing is making its way towards therapeutic applications. However, genome editing tools are usually researched using cancerous, undifferentiated cell lines as experimental models, which do not accurately represent living organisms. SH-SY5Y human neuroblastoma cell line is widely used as a neuronal model, yet in an undifferentiated state that is not representative of mature neurons (Shipley et al., 2016)1. Differentiated SH-SY5Y neurallike cells are distinguished from undifferentiated ones by their neural marker expression, neurite formation and halt of proliferation.

**Aim.** We aimed to create a dual-reporter SH-SY5Y cell line expressing EGFP and mRuby2, which can be used in CRISPR-tools efficiency evaluation at a single-cell scale. Our additional goal was to work out the differentiation conditions allowing fluorescence microscopy.

**Methods.** The dual-reporter EGFP and mRUBY2 system was created by template knockin using CRISPR-mediated HDR into the safe harbour AAV1 locus of SH-SY5Y cells. The donor template and CRISPR-Cas were transfected via lipofection. In the template plasmid, mRuby2 was positioned near its own promoter to be seen after the successful transfection. EGFP, as well as the puromycin resistance gene, was driven by the cell's endogenous promoter, allowing EGFP to be seen only after a successful integration into the genome. The edited cell culture was enriched by puromycin selection. Monoclonal populations of edited cells were obtained by single-cell dilution. Fluorescent cells were identified by fluorescent microscopy. The SH-SY5Y cell line was differentiated using retinoic acid. During later stages of differentiation, cells were grown on the extracellular matrix or Matrigel coating. The Matrigel coating was chosen as an alternative because it allows for fluorescent microscopy.

**Results and conclusions.** We developed a stable SH-SY5Y cell line with reporter proteins EGFP and mRuby2 in the nucleus and membrane, respectively. Throughout 18 days, cells differentiated and began to express neural markers: vesicular glutamate transporter 1 (VGLUT1), post-synaptic density protein 95 (PSD95), and neurofilament marker (SMI312), which were detected using immunofluorescence microscopy. Our choice of Matrigel coating allowed us to inspect the cells using immunofluorescence microscopy.

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## Psychophysical Study of Misperception of Length Caused by a Circular Arcs Distractor

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**Aim:** The aim of the study was to further develop a quantitative model of the filled-space illusion and test it to account for the effects caused by stimuli containing an unconventional form of contextual distractor.

**Methods:** Illusion was measured as a function of the radius of the circular arcs oriented orthogonally and parallel to the main stimulus axis, and the endpoints of imaginary arcs positioned symmetrically with respect to lateral terminator of the three-dot stimulus. Data obtained in different series were fitted with relevant functions of the model. Results: It was shown that the model satisfactorily describes all changes in the illusion magnitude for stimulus with the circular arcs oriented orthogonally and parallel to the main stimulus axis, and the endpoints of imaginary arcs. In addition, it has been demonstrated that the illusion magnitude varies predictably with the size of the arcs central angle.

**Conclusions:** A good correspondence between the experimental and theoretical results supports the suggestion that the context-evoked augmentation of neural excitation can determine the occurrence of the filled-space illusion.

## MIR-23a, MIR-494, MIR-19b, MIR-96 Expression in Parkinson's Disease

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Parkinson's disease is the second leading neurodegenerative disorder in the world that results in bradykinesia and tremor due to the death of dopaminergic neurons. Yet, it is still a challenge to select the best individual treatment and to predict the progression of the disease. Recently, miRNA (microRNA) interference has been extensively studied due to it's affects in many biological processes, including their role in neurodegenerative diseases. MiRNAs originated from extracellular vesicles (EV) are expected to help differentiate diseases, determine their severity and progression, also to select the best treatment option based on individual miRNA profile. The aim of this study was to evaluate EV miR-23a-3p, miR-494-3p, miR-19b-3p and miR-96-5p expression levels in the serum of patients with PD and check for relations with patient data. EV miRNA's were isolated from blood serum, transcribed into cDNA and its expression was measured by RT-PCR. Statistical analysis was performed using GraphPad Software Inc. Prism 8. MiRNA profile was evaluated by age, sex, the onset of the disease, its duration, severity of symptoms and selected method of treatment for 88 individuals with PD. 36 patients received medicational treatment, 39 underwent deep brain stimulation and 13 had gamma knife surgery. The results revealed that patients showed differently expressed miRNA levels when comparing medicational treatment group to surgical treatment groups. MiR-23a-3p expression increased as symptoms of bradykinesia become more severe. On the contrary, miR-494-3p levels were decreasing as Parkinson's symptoms increase, but no statistical significance was observed. Patients age, the onset of the disease and disease duration had no significant influence in miRNA levels. Association of gender and miRNA expression revealed that miR-19b and miR-23a levels were higher in men population. Furthermore, miR-23a and miR-494 expression decreased after gamma knife surgery. However, patients who received deep brain stimulation surgery did not show changes in selected miRNA profile. In conclusion, primary data suggest that different miRNA expression levels show patient heterogeneity and indicate a potential role of miRNA's in PD pathogenesis. However, more research is needed to further evaluate the potential of miRNAs as candidate biomarkers before application in clinical practice.

# SARS-CoV-2 mRNR vaccines induce microglial proliferation in mixed neuronal-glial cultures

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Development of mRNA vaccines was major discovery that helped stop COVID-19 pandemic. All developed vaccines against SARS-CoV-2 showed that they can cause various neurological effects like headache, brain fog and dizziness. Recent research states that mRNA vaccines like Comirnaty (Pfizer) tend to cause side effects after repeated vaccination, though developers did not investigate if there is a direct interaction of vaccine and brain cells. In our study, we aim to investigate whether SARS-CoV-2 mRNA vaccines can directly affect viability of mixed neuronal-glial cells and microglia proliferation. We used primary neuronal-glial cell cultures that we prepared from 6-7 days Wistar rat cerebella. Cell cultures were treated with single injections of 5 different concentrations of Comirnaty (Pfizer) mRNA Original/Omicron B.A. 4-5 and Tozinameran/Riltozinameran vaccines for 3 and 7 days. We also treated cells with three repeated standard 5 ng/ml mRNA vaccine injections every 24 hours incubating for 7 days. Cell viability was evaluated with fluorescent microscope using Hoechst 33342 and Propidium Iodide. Isolectin GS IB4 AlexaFluor 488 was used to identify microglial cells. We showed that single injections of both mRNA vaccines did not affect viability and number of neurons in mixed neuronal-glial cultures after 3 days and 7 days of incubation. After 7-day incubation with singular doses of mRNA vaccines (in the range of 50 ng/ml – 100 ng/ml concentrations) microglial number significantly increased compared to control cultures at the same time while after 3 days there was no effect on microglial number with all concentrations. Repeated injections of standard 5 ng/ ml mRNA vaccines increased microglial number in mixed neuronal glial cultures, but had no significant effect on neuronal number and viability. In conclusion, our data show that mRNA vaccines can induce microglial proliferation, though it does not affect neuronal viability in mixed neuronal-glial co-cultures.

## Hippocampal CA1-CA3 Subfields are Associated with Better Working Memory Capacity

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Evidence suggests that as individuals age, their working memory (WM) capacity tends to decrease (Verhaeghen et al., 2019). Similarly, there is an established link between aging and a reduction in hippocampal volume (Nobis et al., 2019). This raises the question whether there is a direct association between WM capacity and hippocampus. Recent studies indicate that CA1 and CA3 could be related to WM in immediate recall (Zheng et al., 2018), when overlapping stimuli need to be disambiguated (Newmark et al., 2013), in resolution of proactive interference (Andersson et al., 2023) and in encoding (Deuker et al., 2014). To better understand the relationship between the variables, we aim to explore whether hippocampal subfields CA1 and CA3 are related to WM functioning, particularly – WM capacity. Participants: 46 participants, 65 to 85 years (Mage=71.80, SD=5.05, 17,4% male), with no subjective cognitive memory decline.

**Methods:** WM was assessed using the Numbers Reversed test from Woodcock-Johnson Tests of Cognitive Abilities. Structural measures of the hippocampus were obtained using a Siemens 1.5 Tesla Avanto MRI scanner. Volumetric data were extracted using Freesurfer 7.2. software. High-resolution anatomical images of the hippocampus were performed using a 3D T1-weight magnetization prepared rapid Acquisition Gradient Echo (MPRAGE) sequence.

**Results:** Spearman rank correlation indicated statistically significant moderate association between left hemisphere substructures CA1 head (rs = .409, p = .01) and CA3 head (rs = .466, p = .01) and right hemisphere hippocampal CA1 head (rs = .444, p = .01) and CA3 head (rs = .537, p = .01) and WM. CA1 body and CA3 body bilaterally showed no relationship to WM. Furthermore, to examine whether CA1 and CA3 segments of both hippocampal hemispheres explained the WM performance, hierarchal linear regression analysis was conducted, controlling for the estimated intracranial volume. Only CA1-CA3 substructures of the right and left hippocampus were used in the models. The results indicated that the volume of the left hippocampal CA1 head explained 16%, CA3 head explained 25%, but the volume of the right hippocampal CA1 head explained 26%, CA3 head explained 28% of WM.

**Conclusions:** Larger volume of CA1 and CA3 could be associated with better WM performance. The underlying mechanisms may involve an overlap between WM and long-term memory. Further studies in a larger sample are needed, to better understand the relationship.



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