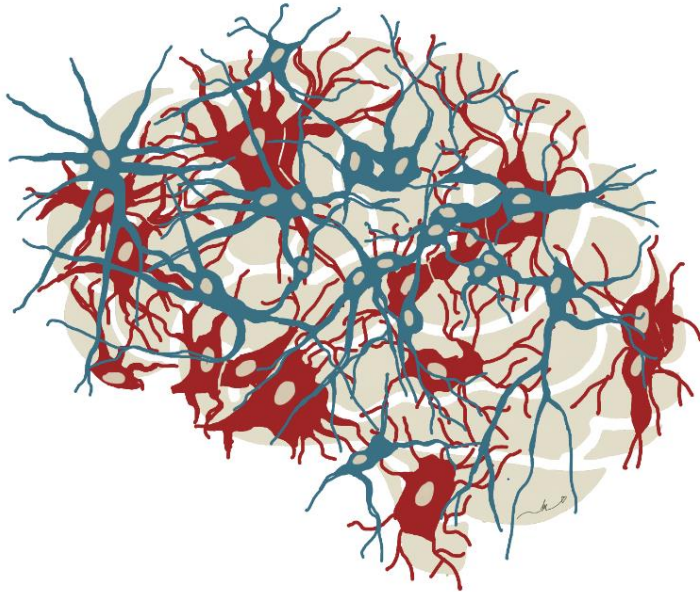**Institute of Neuroimmunology SAS**

ADVANCES
IN EXPERIMENTAL
NEUROIMMUNOLOGY 2023

Smolenice, June 18-20, 2023

ADVANCES IN EXPERIMENTAL NEUROIMMUNOLOGY 2023

Smolenice Castle, Slovakia
June 18-20, 2023

Organized by
Institute of Neuroimmunology Slovak Academy of Sciences

Co-organized with
Slovak Society for Neuroscience
Slovak Society for Immunology

Scientific Programme Committee
Peter Filipcik
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PROGRAM

Sunday	**18 June 2023**	
14:00	***Registration***	
14:55	***Opening the conference*** *Peter Filipcik*	
15:00 – 18:00	***Session I.***	***Neuroscience & immunology: a fruitful alliance***
	Chairs	*Branislav Kovacech, Vladimir Leksa*
15:00	**Shaping Brain Function and Curing Dysfunction by Harnessing the Power of the Immune System**	
	Norbert Zilka	
15:30	**Don't lose your head: Navigating the maze between concussion and Alzheimer's**	
	Igor Jurisica	
16:00	**Modeling naturally occurring diseases in dogs: stem cell-based approach**	
	Dasa Cizkova	
16:30	**A proof of concept: A delivery system to transport drugs across BBB against neuro-pathogens**	
	Mangesh Bhide	
17:00	**General discussion**	
19.00	***Get together party – with participants of InterTAU summer school***	

Monday   19 June 2023

9.00-12.00	**Session II.**	**Novel tools and procedures for understanding of brain**

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Chairs   *Dasa Cizkova, Jozef Hanes*

9:00 **Understanding Brain Disorders**

Tomas Hromadka

9:45 **Application of in situ cryo-ET: Axon branching story**

Hana Nedozralova

10:30 **Coffee break**

11:00 **Blood-brain barrier transport of kynurenines: immunomodulatory and neuroprotective role in the transgenic model for Tauopathy**

Petra Majerova

11:30 **Directed evolution of antibodies for detection of low abundant biomarkers in body fluids**

Jozef Hanes

12:00 **Shifting the detection paradigm for low abundant neurobiomarkers using an ultrasensitive digital immunoassay**

Stanislav Kukla

12:30 – 14:00 **Lunch**

14:00 – 15:45 **Session III.   Neuroimmunology, out of the box**

Chairs   *Rostislav Skrabana, Peter Filipcik*

14:00 **Universal functions of the milk protein lactoferrin – why not in the brain?**

Vladimir Leksa

14:30	**Structural analysis of protein assemblies by solid-state NMR**
	Kristaps Jaudzems
15:30	***Coffee break***
16:00	**Effect of Nonconcussive Repetitive Head Impacts on Tau181/Total Tau in Plasma of Young Elite Soccer Players**
	Martin Cente
16:30	**Heatstroke-induced late-onset neurological deficits in mice caused by Purkinje cell degeneration, demyelination, and synaptic impairment at cerebellum**
	Kazuyuki Miyamoto
17:00	**Stem cell-based therapy for dogs and cats with spinal cord injury**
	Jana Farbakova
17:30	***Concluding remarks and discussion***
19:00	***Social evening – Doľany***

Tuesday   20 June 2023

9:00 – 10:10	***Session IV.***	***Neuroimmune interactions in preclinical models***
	Chair	*Martin Cente*
9:00	**Fluid biomarkers for diagnosis, prognosis of canine neurological disorders, lesson from Human studies**	
	Tomas Smolek	

9:30   **Heavy metals and neurodegenerative diseases:
the response of the rat's pineal gland astrocytic glia
under conditions of long-term influence of heavy metal
salt's mixture and different periods of adaptation**

Natalia Hryntsova

10:00   **Coffee break**

10:15 – 12:20   **Session V.**   **Selected presentations
of doctoral students at the
Institute of Neuroimmunology**

Chairs   *Mangesh Bhide, Tomas Hromadka*

10:15   **Identification of tau interacting proteins
and associated molecular pathways in vivo**

Jakub Sinsky

10:25   **miRNAs as primary molecular players in repetitive
head impacts – association with signaling pathways leading
to neurodegeneration**

Sara Porubska

10:35   **Debunking the Myth: Chronic Inflammation
Might Not Be Fueling Tau Pathology**

Neha Basheer

10:45   **Mechanism of tau protein transport
through the Blood-CSF barrier**

Krutika Khiratkar

10:55   **The association between cellular senescence and progression
of neurodegenera-tive diseases: From traditional marker
screenings to the generation of novel senescence-reporting
neuronal cell lines**

Kristina Macova

11:05	**Effect of AD-Tau aggregation on mitochondria in Tau FRET biosensor cell model**
	Muhammad Khalid Muhammadi

11:15	***Coffee break***

11:30	**The interconnection of senescence and alpha-synuclein related pathologies in neurodegeneration**
	Miraj Ud Din Momand

11:40	**Construction of CDR3-phage library for selection of neutralizing peptides against tick-borne encephalitis virus**
	Tomas Malarik

11:50	**Animal models of TBI, first experience at ISNI**
	Marian Horvath

12:00	**Investigation of metastable conformations of the neuronal protein Tau to design novel anti-AD drug candidates**
	Stefana Njemoga

12:10	**Mesenchymal stem cell therapy for the treatment of traumatic brain injury**
	Andrej Durgala

12:20	***Concluding remarks – end of the conference***

The posters will be displayed during entire duration of the conference in front of the lecture hall.

Discussion time: *Monday*   *8:30 – 9:00*

Katarina Bhide   **Domain III of envelop glycoprotein of West Nile virus (WNV) affects signalling events in human brain microvascular endothelial cells**

Jana Hruskovicova   **Development of nanobody-based nanocarrier system against tick-borne encephalitis virus to overcome the blood-brain barrier**

Kevin James   **Production of anti-dengue aptamers using dengue virus like particles**

Katarina Kuckova   **Dendrimer as an antimicrobial agent against Neisseria meningitidis**

Lea Talpasová   **Synthesis of structurally constrained CDR3 peptides against SARS-CoV-2**

ABSTRACTS

SHAPING BRAIN FUNCTION AND CURING DYSFUNCTION BY HARNESSING THE POWER OF THE IMMUNE SYSTEM

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The immune system, traditionally associated with defence against pathogens and cancer cells, also exerts a profound influence on brain function. In addition to safeguarding the brain, the immune system actively participates in shaping brain development, synaptic plasticity, and neuronal communication. Microglia along with peripheral leukocytes constantly survey the microenvironment of the brain, responding to neuronal activity and immune signals. Their intricate interactions with neurons and other glial cells are crucial for maintaining brain homeostasis and modulating synaptic connections. In addition, immune cells can infiltrate the brain during injury or disease, mounting an immune response that influences neuronal survival and tissue repair. The immune system also plays a role in neuroinflammation, which, when dysregulated, can contribute to neurodegenerative disorders and cognitive decline. Furthermore, recent studies highlight the impact of the gut microbiota on immune-brain interactions, suggesting a bidirectional communication pathway that can influence brain health and function. Indeed, the recent breakthroughs in immunotherapy for Alzheimer's disease illuminate the possibility of combating fatal neurological disorders by employing advanced immunological interventions.

DON'T LOSE YOUR HEAD: NAVIGATING THE MAZE BETWEEN CONCUSSION AND ALZHEIMER'S

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Integrative computational biology and artificial intelligence help improving treatment of complex diseases by identifying biomarkers and building explainable models characterizing individual patients. From system-atic data analysis to more specific diagnostic, prognostic and predictive biomarkers, drug mechanism of action, and patient selection, such analyses influence multiple steps from prevention to spectrum of disease characterization, and from prognosis to drug discovery. To fathom complex biological processes both in healthy and disease states, we need to systematically integrate multi-omics datasets, ontologies and diverse annotations. Without proper management of such complex biological and clinical data, artificial intelligence algorithms cannot be effectively trained, validated and successfully applied. Despite challenges, there are many opportunities in precision medicine using AI, big data analytics and integrative computa-tional biology workflows. Data mining, machine learning, graph theory and advanced visualization help identify diagnostic, prognostic and predictive biomarkers, and create causal models of disease. Intertwining computational prediction and modelling with biological experiments leads to faster, more biologically and clinically relevant discoveries.

MODELING NATURALLY OCCURRING DISEASES IN DOGS: STEM CELL-BASED APPROACH

D. Cizkova[1,3], J. Farbakova[2], M. Kuricova[2], N. Hudakova[2], L. Hornakova[2],
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Naturally occurring diseases in dogs are similar in many aspects to those found in humans. From this perspective, the inclusion of spontaneously developed canine diseases in the development of innovative therapies based on mesenchymal stem cells (MSCs) can significantly benefit human clinical trials. MSCs originating primarily from bone marrow (BM-MSCs), adipose tissue (AT-MSCs), umbilical cord tissue (UC-MSCs), or amnion (AM-MSCs) of healthy dogs are among the most intensively studied cells with wide clinical application in current veterinary medicine. Their application requires standardized, evidence-based protocols as well as well-characterized MSCs fulfilling criteria for mesenchymal proper-ties. However, the therapeutic effect of MSCs is often mediated by their paracrine function via the se-cretome and extracellular vesicles (EVs) released from the cells. Therefore, an alternative upcoming cell-free strategy has been directed toward MSCs secretome or EVs-related therapies. Although they are gaining dominant attention for treating common diseases in dogs, more studies need to be conducted in compliance with the functional principle, bio-availability, defined dosage, and administration. It is im-portant to clarify the current state of MSCs-based treatment in the field of neurodegenerative disorders, osteochondral diseases, or chronic kidney failures, their challenges, limitations, and possible impacts on human medicine.
Furthermore, pet dogs with spontaneous cancer (mammary gland tumors/MGT) are also important translational models that can be incorporated into the development of early-stage biomarkers. Particular-ly, the changes in intracellular microRNAs (miRNAs) within the tissue and free miRNAs in biofluids (blood plasma, urine) may outline the most significant changes between healthy and tumor patients and may be useful for comparative analysis of their expression before and after treatment.

A PROOF OF CONCEPT: A DELIVERY SYSTEM TO TRANSPORT DRUGS ACROSS BBB AGAINST NEURO-PATHOGENS

M. Bhide[1,2], A. Kulkarni[1,2], P. Majerová[2], E. Mochnáčová[1], K. Bhide[1], J. Hruškovicová[1], K. Kucková[1], K. James[1,2], T. Maľarik[1], L. Talpašová[1], A. Kováč[1,2]

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Infectious diseases affecting the central nervous system (CNS) remain an important source of morbidity and mortality. A major obstacle for curing brain diseases is the blood-brain barrier (BBB), which im-pedes therapeutic agents to reach the brain and target the related pathogens. The aim is to develop a drug delivery nanosystem coated with antibody fragments, called nanobodies (Nbs) as a proof of concept for targeting brain infections caused by bacteria and virus. To do this, nanobodies (Nbs) will be generat-ed against neurotropic pathogens against two bacterial (Neisseria meningitidis and Borrelia burgdorferi), and three virial pathogens (TBEV, WNV and SARS-CoV-2). The so-created Nbs will be then conjugated to the drug-loaded nanoparticles constructed using dendrimer nanovectors. The obtained nanodrug can-didates will also contain Angiopep2 BBB homing peptide. Nanocarrier system will be character-ized for their size, morphology, surface charge and stability as well as drug loading and release profile etc. The ability of these nanosystems to across BBB will be assessed us-ing an in vitro model of BBB, and their preclinical safety and biological activity against the neurotropic pathogens will be assessed in vitro using cell based experiments and in vivo using animal models. The success of this project will validate the proof-of-concept study to combine the nanobody technology with the nanotechnology based drug deliv-ery for effectively overcoming BBB and targeting pathogens in brain infec-tions. We expect to generate, in this project, clinical useful pilot results for the best performing candidates for future translation, and at the same time, research data of general scientific interest useful to the broad scientific community.

APPLICATION OF *IN SITU* CRYO-ET: AXON BRANCHING STORY

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In situ cryo-electron tomography is an emerging imaging technique able to visualize cellular features in a near-native environment at nanometre resolution. In contrast to electron microscopy methods used for biology, vitrified cryo-ET samples do not suffer from the artefacts introduced by the traditional sample fixation and the advance in focus ion beam milling technology now allows the preparation of thin sample sections (or lamella) without the deformations of mechanical microsectioning.

This presentation aims to introduce the methodology of cryo-ET for cellular biology purposes with a focus on neurobiology application which will be demonstrated in the study of axon branching [1].

Axon branching is an important process in neuron development. However, our understanding of branch formation is sparse due to the lack of direct in-depth observations. Using in situ cellular cryo-ET on primary mouse neurons, we directly visualized the remodeling of organelles and cytoskeleton structures at axon branches.

1. H. Nedozralova, N. Basnet, I. Ibiricu, S. Bodakuntla, C. Biertümpfel, N. Mizuno, J Cell Biol., 2022 Apr 4;221(4):e202106086. doi: 10.1083/jcb.202106086.

BLOOD-BRAIN BARRIER TRANSPORT OF KYNURENINES: IMMUNOMODULATORY AND NEUROPROTECTIVE ROLE IN THE TRANSGENIC MODEL FOR TAUOPATHY

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The blood-brain barrier (BBB) represents the bottleneck in brain drug development and is the single most important factor limiting the future growth of neurotherapeutics. Essentially 100% of large-molecule pharmaceutics, including peptides, recombinant proteins, monoclonal antibodies, RNA interference (RNAi)-based drugs and gene therapies and >98% of small-molecule drugs do not cross the BBB. Tauopathies are a heterogeneous group of neurodegenerative disorders, pathologically characterized by neuronal and/or glial intracellular inclusions of the microtubule-binding protein, tau. The pathogenesis of tauopathies has been associated with systemic inflammation and disruption of the serotonergic signaling system. A common link between neuroinflammation and disruption of the serotonergic signaling system is the catabolism of the essential amino acid- L- tryptophan (L-TRP). Levels of kynurenines can be altered in aging and neuropathological conditions leading to decreased biosynthesis of nicotinamide. Several studies indicate the significant involvement of the kynurenine pathway (KP) in the pathogenesis of Alzheimer´s disease (AD). Kynurenines exert immunomodulatory and neuroactive properties and can influence the central nervous system. However, neuroprotective kynurenic acid has limited ability to cross the BBB, therefore different analogs are necessary to exploit its therapeutic potential. In the present study, we used a modified synthesized neuroprotective derivate of kynurenic acid N-(2-N, N-dimethylaminoethyl)-4-oxo-1H-quinoline-2-carboxamid (KYNA-1) to modulate neurofibrillary pathology in the brain. The analog was intraperitoneally administrated into a transgenic rat model for tauopathies. KYNA-1 treatment induced a decrease in glial fibrillary acid protein (GFAP) levels and a reduction in sarkosyl-insoluble tau. We found a significant dose-dependent reduction in GFAP levels after KYNA-1 treatment.

DIRECTED EVOLUTION OF ANTIBODIES FOR DETECTION OF LOW ABUNDANT BIOMARKERS IN BODY FLUIDS

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Alzheimer's disease (AD) is a neurodegenerative brain disorder in which progressive dementia occurs. The causes are still not clear. To date, there is no cure for AD and no FDA-approved biomarker. We developed a novel pT217 tau ELISA assay, which measures phosphorylated tau proteins on Thr217 in cerebrospinal fluid [1]. We showed that this novel assay can distinguish AD from healthy individuals and AD from other neurodegenerative diseases with very high sensitivity and specificity and is more sensitive and specific compared to other currently used biomarkers. Because this assay was not sensitive enough to measure pT217 tau species in blood we improved the affinity of pT217-tau binding antibody by ribosome display. In collaboration with our partner, we developed a pT217 assay for blood and showed that it was possible to measure pT217 tau in all AD and control plasma samples.

1.   Hanes, J., Kovac, A., Kvartsberg, H., Kontsekova, E., Fialova, L., Katina, S., Kovacech, B., Stevens, E., Hort, J., Vyhnalek, M., Boonkamp, L., Novak, M., Zetterberg, H., Hansson, O., Scheltens, P., Blennow, K., Teunissen, C.E. and Zilka, N. (2020) Evaluation of a novel immunoassay to detect p-Tau Thr217 in the CSF to distinguish Alzheimer disease from other dementias. Neurology 95:e3026-e3035

UNIVERSAL FUNCTIONS OF THE MILK PROTEIN LACTOFERRIN – WHY NOT IN THE BRAIN?

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Lactoferrin, a member of the lactotransferrin family of iron-binding glycoproteins, is present in most human exocrine fluids, particularly mother milk. Both human and bovine lactoferrin exhibit a plethora of biological activities, including an iron seques-tration, a blockade of proteases, or a direct modulation of immune cells. Via these activities lactoferrin plays manifold roles in antimicrobial host defense. Fur-thermore, antitumor functions have also been attributed to lactoferrin. The ingested lactofer-rin is cleaved upon digestion in the gastrointestinal tract, yielding bioactive peptides called lactoferricins and lactoferrampins, which preserve and even augment some activities of the intact protein. Altogether, these properties make lactoferrin a cheap and widely available candidate for supplementary therapy in management of infec-tious diseases, including COVID-19. Here, I will focus on the role of lactoferrin in regu-lation of pericellular proteolysis and discuss its possible implications in neurobiology.

STRUCTURAL ANALYSIS OF PROTEIN ASSEMBLIES BY SOLID-STATE NMR

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The atomic-level characterization of non-diffracting protein assemblies such as fibrillar aggregates and viral capsids is one of the greatest challenges of modern structural biology, as well as a fundamental step for the design of effective treatments. Over the last decades, solid-state NMR (ssNMR) has developed into a powerful structural tool for studying structure and dynamics of solid biological samples at atomic resolution. However, the inherently low sensitivity and poor resolution of the technique has limited its applicability to small proteins that can be tightly packed at a high molar concentration, while large proteins or multi-domain assemblies were mostly inaccessible to site-specific ssNMR studies. This has been recently overcome by the introduction of faster spinning probes, which facilitate the use of proton-detected ssNMR experiments [1], as well as by dynamic nuclear polarization (DNP), which allows transfer of polarization from the unpaired electrons of a paramagnetic center to the surrounding nuclei, and can enhance the sensitivity of ssNMR experiments by several orders of magnitude [2-3]. In my presentation, I will provide an overview of state-of-the-art ssNMR techniques for structural biology applications as well as show examples of ssNMR and DNP structural studies of different non-diffracting samples [4-6].

1. T. Le Marchand, T. Schubeis, M. Bonaccorsi, P. Paluch, D. Lalli, A.J. Pell, L.B. Andreas, K. Jaudzems, J. Stanek, G. Pintacuda, *Chem. Rev.*, 122, (2022), 9943.
2. K. Jaudzems, A. Bertarello, S.R. Chaudhari, A. Pica, D. Cala-De Paepe, E. Barbet-Massin, A.J. Pell, I. Akopjana, S. Kotelovica, D. Gajan, O. Ouari, K. Tars, G. Pintacuda, A. Lesage, *Angew. Chem. Int. Ed.*, 57, (2018), 7458.
3. K. Jaudzems, T. Polenova, G. Pintacuda, H. Oschkinat, A. Lesage, *J. Struct. Biol.*, 206, (2019), 90.
4. M. Otikovs, M. Andersson, Q. Jia, K. Nordling, Q. Meng, L.B. Andreas, G. Pintacuda, J. Johansson, A. Rising, K. Jaudzems, *Angew. Chem. Int. Ed.*, 56, (2017), 12571.
5. K. Jaudzems, A. Kirsteina, T. Schubeis, G. Casano, O. Ouari, J. Bogans, A. Kazaks, K. Tars, A. Lesage, G. Pintacuda, *Angew. Chem. Int. Ed.*, 60, (2021), 12847.
6. J. Fridmanis, Z. Toleikis, T. Sneideris, M. Ziaunys, R. Bobrovs, V. Smirnovas, K. Jaudzems, *Int. J. Mol. Sci.*, 22, (2021), 9635.

EFFECT OF NONCONCUSSIVE REPETITIVE HEAD IMPACTS ON TAU181/TOTAL TAU IN PLASMA OF YOUNG ELITE SOCCER PLAYERS

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Head impacts resulting in traumatic brain injury (TBI) are a significant risk factor for the development of sporadic tauopathies such as chronic traumatic encephalopathy, Alzheimer's disease, and other neurocognitive disorders. However, the data on the consequences of low-intensity repetitive head impacts that are not followed by loss of consciousness (nonconcussive head impacts) are inconsistent. Therefore, it is not clear whether or not such mild head impacts are associated with neuropathology.

In this study, we investigated the effect of heading in soccer on the plasma level of total and phosphorylated tau protein (p-tau181). The experimental cohort included young elite soccer players who performed intense physical activity with and without heading the ball.

The primary study outcomes were the levels of total tau protein and p-tau181 in plasma samples and the cognitive status of the study participants (n=37).

We found significantly elevated levels of total tau and p-tau181 in the plasma of soccer players 1 hour after physical exercise (tau, 1.4-fold; $P < .001$; p-tau181, 1.4-fold; $P < .001$) and repetitive head impacts (tau, 1.3-fold; $P < .001$; p-tau181, 1.5-fold; $P < .001$). The ratio of p-tau181 to tau was significantly higher 1 hour after exercise and heading training, and remained elevated specifically in the heading group even after 24 hours (1.2-fold; $P = .002$). Performance in cognitive tests revealed a signifi-

cant decline in focused attention and cognitive flexibility after physical exercise and heading training, while the physical exercise of higher intensity without heading training was associated with a greater negative cognitive performance than heading only. The increase of p-tau181 levels relative to tau after 24 hours indicates an acute enrichment of phosphorylated tau fraction in the periphery of head impacted individuals. These findings are potentially relevant for the assessment of the consequences of nonconcussive repetitive head impacts, such as heading in soccer, in association with an increased risk of developing neurodegenerative disorders later in life.

HEATSTROKE-INDUCED LATE-ONSET NEUROLOGICAL DEFICITS IN MICE CAUSED BY PURKINJE CELL DEGENERATION, DEMYELINATION, AND SYNAPTIC IMPAIRMENT AT THE CEREBELLUM

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Global warming increases the incidence and severity of heatstroke near future [1]. After a heatstroke, patients who exhibit certain neurological symptoms, such as disorientation, wobbling, and vertigo—are bedridden, thereby preventing patients from returning to normal life, and suggesting cerebellar damage [2]. Moreover, several interesting reports showed that neurological deficits appeared several weeks after heatstroke rather than immediately afterward [3], and some cases improved with time [4], while others are permanent. However, it is not well understood how these late-onset neurological deficits post-heatstroke appear and, in some cases, improve over time. Therefore, we focused on the influence of heatstroke on the cerebellum using the mice heatstroke model. In our study, motor coordination disorder significantly appeared 3 weeks post heatstroke and gradually improved to some extent. Demyelination was detected at 1 and 3 weeks after heatstroke in the cerebellum and improved in 9 weeks. It was not found in the corpus callosum. The Purkinje cell numbers significantly decreased at 1-, 3-, and 9-weeks post heatstroke. The intensity of synaptophysin and postsynaptic density-95 temporarily appeared to attenuate at 3 weeks and recovered at 9 weeks post heatstroke. Our results suggested that permanent Purkinje cells loss, transient demyelination and synaptic impairment at cerebellum might correlated with delayed cerebellar ataxia of heatstroke. In further, we will investigate the mechanism of the neurodegeneration after heatstroke to investigate the new treatment.

1. Meehl, G. A. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 2004, 305, 994-997.
2. Lawton, E. Review article: environmental heatstroke and long-term clinical neurological outcomes: A literature review of case reports and case series 2000-2016. Emerg. Med. Australia 2019, 31, 163-173.
3. Jung, I. Delayed vestibulopathy after heat exposure. J. Neurol 2017, 264, 49-53.
4. Lo, Y. Diffuse cerebral cortex, cerebellar cortex and basal ganglia injury: A rare MR imaging manifestation of heat stroke. Neuroradiol. J 2015, 20, 37-40.

STEM CELLS BASED THERAPY FOR DOGS AND CATS WITH SPINAL CORD INJURY

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Neurological diseases resulting from damage to the spine and spinal cord are among the most common in the clinical practice of small animals. They are often caused by numerous external and internal fac-tors. For animals and their owners, spinal cord injuries (SCI) are often frustrating, and they are also as-sociated with an unfavourable prognosis in terms of recovery of the function in the affected limb or body part. In dogs, SCI is usually presented with sensory and motor deficits accompanied by ataxia, pain, paresis or paralysis, and urinary disturbances. Despite the large number of treatment strategies used in experimental and clinical settings, prognosis and outcome after SCI are still largely unpredicta-ble in animals and people, as well. Prognosis is primarily related to early and correctly selected diagnos-tics and subsequent adequate therapy. In addition, the final prognosis is influenced by factors such as the cause and strength of the traumatic spinal cord injury, the time interval from the trauma to the cor-rectly selected therapy, and the overall management of the neurological patient in the preoperative and postoperative periods.

In our clinical research, we are dealing with the possibility of improving the prognosis in such affected dogs and cats by applying therapy based on stem cells. The ideal therapy is based on the local applica-tion of a conditioned medium derived from stem or progenitor cells of mesenchymal and neural origin. In the case of spinal cord involvement, our route is a combination of intravenous, intrathecal and epi-dural administration to maximize the potential effect of this stem cell product. During the study, we focus on the evaluation of the clinical neurological status through the neurological score, the measure-ment of muscle group circumferences, the monitoring of urinary function, and blood parameters.

This therapy appears to be very promising, in safety studies, we have not reported the occurrence of serious adverse side effects, in clinical studies, we have achieved posi-tive (yet unpublished) results after spinal cord trauma in dogs and cats.

FLUID BIOMARKERS FOR DIAGNOSIS, PROGNOSIS OF CANINE NEUROLOGICAL DISORDERS, LESSON FROM HUMAN STUDIES

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The cerebral biomarkers, neurofilament light chain (NfL), amyloid β, tau, and neuron specific enolase (NSE) are widely used for detection of neurological damage in the brain and spinal cord. With this study, we aimed to assess whether these biomarkers hold any potential diagnostic value also for the three most common canine neurological diseases.

Canine suffering from meningoencephalitis with unknown origin (MUO), brain tumors, and selected non-infectious myelopathies were included in this study. We analysed these biomarkers for each diagno-sis in the cerebrospinal fluid by ELISA. CSF was collected by the cranial puncture from cisterna magna.

We observed significant increase in the levels of CSF tau, NfL, and NSE in MUO. A significant correla-tion between these three biomarkers was identified. Tau and NSE was increased but amyloid β was de-creased in dogs suffering from tumors. Interestingly, none of the biomarkers was changed in dogs with myelopathies. Minimal effect of covariates (age, sex or castration) was observed.

We showed that the biomarkers in CSF may reflect molecular changes related to MUO and tumors but not for non-infectious myelopathies. Combination of NfL, tau and NSE may represent ideal biomarkers for MUO because they reflect the same pathology and they are not influenced by age.

HEAVY METALS AND NEURODEGENERATIVE DISEASES: THE RESPONSE OF THE RAT'S PINEAL GLAND ASTROCYTIC GLIA UNDER CONDITIONS OF LONG-TERM INFLUENCE OF HEAVY METAL SALT'S MIXTURE AND DIFFERENT PERIODS OF ADAPTATION

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Abstract: Excessive intake of heavy metals, such as As, Mn, Hg, Al, Pb, Ni, Bi, Cd, Cu, Zn, Fe is neurotoxic and it promotes neurodegeneration. Astrocytes protect neurons against all types of insults, in particular by accumulating heavy metals. The neurotoxicity caused by heavy metals in astrocytes can play a deteriorative role in facilitating neurodegenerative diseases [1]. The combined effect of 4 or more heavy metals causes more serious neurotoxic effects compared to those caused by mixtures of two or three toxic metals [2]. Melatonin protects neurons against death induced by oxidative stress and Ab toxicity [3]. Pineal dysfunction and reduced melatonin levels are directly related to the pathological progression of AD [4]. However, the mechanisms connecting pineal gland dysfunction and AD pathologies are not fully understood.

The experiment was performed on 72 white sexually mature male rats, aged 7–8 months, which were divided into 6 control and 6 experimental ones. The experimental groups 1 – 4 included rats, which for 30, 60 and 90 days received drinking water with a mixture of heavy metal salts: Zn, Cu, Fe, Mn, Pb, Cr. The experimental groups 5-6 consisted of rats in the adaptation period, which for 30-, 90 days were on the usual drinking and food ration after 90 days of exposure to a mixture of heavy metal salts. We used general histological research method (hematoxylin-eosin), morphometry and statistical methods.

During the 30-day period of the experiment, an active adaptive response of neuroglia to the action of heavy metal salts was observed. The absolute number of astrocytes increased by 1.4 times (P>0.05) compared to the control. On the 60th day, a rather pronounced active glial reaction was detected in the form of reactive astrogliosis, both local (around the vessels) and diffuse. On the 90th day of the experiment, the manifestations of reactive astrogliosis are most pronounced. Focal accumulations of astrocytes with the formation of glial nodules were found subcapsular around vessels with impaired permeability of the vascular wall and hemorrhages. In addition to the local, general diffuse glial reaction was preserved. The absolute number of astrocytic glia both on the 60th and 90th day of the experiment increased by 2.2 times (P<0.001, t=13.8) compared to the control. During the 30-day adaptation period, the activity of astrocytic glia increased, especially around pathologically

changed vessels, microhemorrhages, in the growth zones of the stroma and at the site of apoptically changed pinealocytes with the formation of a moderate number of small cysts. On the 90th day of adaptive changes, a decrease in the intensity of the diffuse glial reaction was found, which was explained by the disappearance of the effect of the stress agent on the organ. However, in some subcapsular parts of the organ, single small glial nodules are still visualized. The absolute number of astrocytes in the epiphyses of the experimental animals increased by 2.6 times (p≤0.001, t=7.81) during the 30-day adaptation and by 1.8 times (p≤0.001, t=4.84) during the 90-day adaptation adaptation compared to the control. Thus, the effect of a mixture of heavy metal salts on the pineal gland causes an active glial reaction in the form of diffuse and local reactive astrogliosis. The 30- and 90-day period of adaptation to long-term exposure to heavy metals was insufficient to completely neutralize the effects of microelementosis, eliminate astrogliosis, and achieve homeostasis in the pineal gland.

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IDENTIFICATION OF TAU INTERACTING PROTEINS AND ASSOCIATED MOLECULAR PATHWAYS *IN VIVO*

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Tauopathies, the most representative among which is Alzheimer's disease (AD), are currently nearly incurable neurodegenerative diseases characterized by tau protein pathology [1]. Tau protein, which is mainly present in neurons, plays several physiological functions under normal conditions, such as stabilization of microtubules, participation in the regulation of synaptic plasticity, or protective interactions with DNA and RNA [2,3]. In AD and other tauopathies, tau protein undergoes pathological modifications that lead to the several pathological processes in neurons and the brain, such as its self-aggregation, disruption of intracellular transport and protein homeostasis, neuronal death, and chronic inflammation [4,5]. Worldwide, an increasing number of patients are suffering from AD, and although several therapeutic approaches have been developed to target tau pathology, there is still no reliable treatment [6]. Since formation of protein complexes and protein-protein interactions (PPIs) are the fundamental pillars for molecular pathways and all biological processes, disruption of the physiological interactome of the tau protein is likely the cause of the development of pathological forms of tau [7]. The relevance of this topic is evident from the number of known tau interacting proteins (TIPs), which have been more than doubled in the last 5 years [8]. The yet known TIPs offers a lot of opportunities for the investigation of molecular pathways affecting tau pathology. However, the number of studies that confirmed TIPs in vivo is limited. The majority of studies reporting TIPs used conventional approaches of molecular biology, which, despite the great robustness, are not capable to capture weak and transient PPIs. Because also weak and transient PPIs can have a substantial effect on cellular signalling and processes, there is a growing need to identify those TIPs using biochemical approaches competent to capturing also weak and transient interactions, such as chemical crosslinking [9], proximity-labelling [10], FRET [11] and other biophysical methods. Researchers at the Institute of Neuroimmunology developed unique transgenic animal models with tau pathology similar to Alzheimer's disease [12–15]. We used one of these models, namely the SHR72 rat, for the identification of novel TIPs. We applied in vivo crosslinking strategy, where

both strong and weak interactions could be identified in the brains of SHR72 rats. After identification of purified TIPs, we validated them using coimmunoprecipitation and colocalization experiments in animal and cellular models. Furthermore, bioinformatic analysis revealed several associated molecular pathways which may play a role in tau pathology.

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miRNAs AS PRIMARY MOLECULAR PLAYERS IN REPETITIVE HEAD IMPACTS – ASSOCIATION WITH SIGNALING PATHWAYS LEADING TO NEURODEGENERATION

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Traumatic brain injuries (TBI) belong to major health problem in contemporary society. From the total number of TBI cases, up to 90% represent mild TBI, while the most at-risk groups include children, people of productive age, and athletes actively practicing in contact sports, who are repeatedly exposed to head impacts of various intensities without any external head protection during training and matches. The association between this kind of head injury and the later development of neurodegeneration, especially chronic traumatic encephalopathy (CTE) has already been described. However, the potential connection between sub-concussive head impacts and the later development of neurodegeneration has not been studied yet. Although, there are many examples of contact sport players who suffered neuropsychiatric disorders eventually neurodegeneration after retirement. Here we have decided to study the effect of non-concussive repetitive head impact in soccer players via analyzing their peripheral blood.

In our study, we focused on the identification of molecular mediators and peripheral biomarkers induced by mild non-concussive head impact. We have collected blood samples from 46 soccer players, students of the Faculty of Physical Education and Sports, Comenius University, in Bratislava. Participants underwent two types of training, heading and identical physical exercise without heading as a control experiment. Timepoints for blood withdrawal were set before the training session, 1 hour, and 24 hours after the training. Using qRT-PCR arrays, we identified a set of 117 unique deregulated microRNAs as primary biomarker candidates after the training sessions. Out of these primary hits, 37 molecules were validated using qRT-PCR assays in the large cohort of subjects. The panel of 33 significantly deregulated miRNAs we used as entry data for a comprehensive and integrative computational biology platform analysis with a focus on the identification of neuro-associated and recovery pathways in the acute phase after RHIs.

The validated miRNAs were also used in the correlation analysis with results of the previous work, obtained in collaboration with our research team[1], to which miRNA analyses followed. We identified eight miRNAs which profile correlated with Tau protein levels, p-Tau181 ratio, percentage of maximum heart rate, results of TMT-B cognitive testing, and a number of headers during the training.

Understanding the deregulation of molecular pathways caused by RHIs may help elucidate not only pathogenic mechanisms after head trauma but also the mechanisms that can lead to spontaneous recovery after mild head injuries.

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DEBUNKING THE MYTH: CHRONIC INFLAMMATION MIGHT NOT BE FUELLING TAU PATHOLOGY

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Alzheimer's disease (AD) is a complex disorder neuropathologically defined by amyloid plaques, cerebral amyloid angiopathy, and neurofibrillary pathology [1, 2]. Further, it's associated with astrogliosis and microglial activation, hinting at a neural immune response [3]. Chronic inflammation, a defining feature of AD, plays a pivotal role in its pathogenesis and progression, with neuroinflammation displaying potential for both beneficial and detrimental effects. Furthermore, individual differences in neuroinflammatory status can uniquely impact tau pathology. Our study explored the effects of chronic systemic inflammation on tau pathology in the R3m/4 mouse model of AD, a model expressing truncated tau (151–391/3R) found in early-stage AD and presenting with brainstem tangle-like pathology. Chronic immune system activation was achieved using bacterial endotoxin (lipopolysaccharide, LPS). Our findings demonstrated a notable decrease in hyperphosphorylated tau at AD-specific epitopes in the hippocampus, and tangle pathology in the brainstem. These results lend credence to the hypothesis that chronic neuroinflammation can differentially influence AD pathology. The role of neuroinflammation in AD progression should therefore be considered contextually and not as a uniform phenomenon.

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MECHANISM OF TAU PROTEIN TRANSPORT THROUGH THE BLOOD-CSF BARRIER

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Tauopathies are referred as the pathological aggregation of tau proteins in the brain in various neurodegenerative diseases. The blood-cerebrospinal fluid (BCSF) barrier is one of the most important barriers in the central nervous system. The Tau proteins have been found in the extracellular space before the neuronal death which indicates its secretion by neuronal cells. This project aims to study the molecular and cellular mechanism of efflux of the tau proteins to and from the brain using the BCSF barrier which was developed by using the primary choroid plexus epithelial cells. The in vitro results showed that the proline-rich region containing tau proteins has highest transportation rate. The findings were confirmed by the in vivo study in SHR24 transgenic rats that showed the interaction of N-terminal and proline rich region tau protein with the choroid epithelial cells. The data confirms the role of BCSFB and tau proteins and its active transportation across the barrier.

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THE ASSOCIATION BETWEEN CELLULAR SENESCENCE AND PROGRESSION OF NEURODEGENERATIVE DISEASES: FROM TRADITIONAL MARKER SCREENINGS TO THE GENERATION OF NOVEL SENESCENCE-REPORTING NEURONAL CELL LINES

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In the last few decades, the average age of populations, especially in developed countries, has notably increased. This seemingly positive result of an improved healthcare system and better living conditions has, however, led to a rising prevalence of age-related pathologies, including metabolic disorders, cardiovascular diseases, cancer, and neurodegeneration. Despite being very heterogeneous in the sense of initial cause and manifestations, the majority of these chronic conditions is accompanied by a common biological process called senescence [1, 2].

Although primarily recognized as an essential tumor-suppressor mechanism that prevents the proliferation of damaged and potentially malignant cells, persisting senescence has been linked to the onset

and/or progression of multiple neurodegenerative diseases (NDs), such as the second most common ND, Parkinson's disease (PD) [1, 2]. Moreover, despite the initial association only with proliferating cells, groundbreaking discoveries revealed that even post-mitotic fully differentiated neurons can develop

a senescent-like phenotype (e.g. increased senescence-associated β galactosidase (SA-β gal.) activity; elevated levels of cyclin-dependent kinase inhibitors p16INK4a and p21Waf1/Cip1, pro-inflammatory molecules, and anti-apoptotic proteins) [2, 3]. However, it is not clear yet (i) whether the load of senescent cells in an organism correlates with the stage of NDs and (ii) if elevated levels of these features, specifically in neurons, are involved in the pathogenesis of chronic conditions.

Therefore, the aim of our research is to establish a new approach of analysis and address these crucial

yet unresolved questions. Firstly, we analyze the correlation between senescence induction and progression of PD pathology in human patients. We are using different screening methods (e.g. qPCR, WB, ICC)

to monitor and quantify expression levels of the main senescence hallmarks (e.g. SA-β gal. activity, p16INK4a, p21Waf1/Cip1) initially in peripheral tissues and later on in post-mortem brains. In parallel, to analyze

the neuronal senescence-like response, we successfully developed previously missing molecular tools that can be stably integrated into the genome of various cell types, including human neurons, to report senescence induction. These DNA constructs, created by molecular cloning, consist of a reporter gene (encoding either a red fluorescence protein Tomato or a luciferase NanoLuc) placed in between two 500-650 bp long sequences amplified from either p16INK4a or p21Waf1/Cip1 alleles. By employing the CRISPR/Cas9 targeting technique, we knocked-in our donor constructs into the genome of human neural progenitor ReNVM cells to generate novel senescence-reporting cell lines. These genome-modified cells are being used to induce neuronal senescence and analyze the features and consequences of the resulting phenotype. Subsequently, we sought to identify the main regulators of this process by modifying the expression
of various senescence-associated genes.

Thus far, our preliminary results revealed elevated levels of several senescence markers (e.g. SA-β gal. activity, p16INK4a, and p21Waf1/Cip1) in fibroblasts from PD patients compared to controls, and initial trials
of senescence induction in generated senescence-reporting neural cell lines resulted in several cells positive for Tomato fluorescence. We believe that our progressing research can help to clarify the yet unknown manifestations, and consequences of neuronal senescence-like response and evaluate the role of this process in PD.

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EFFECT OF AD-TAU AGGREGATION ON MITOCHONDRIA IN TAU FRET BIOSENSOR CELL MODEL

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Alzheimer's disease (AD) is the most commonly occurring neurodegenerative disorder. It is characterized by the accumulation of amyloid β (Aβ) plaques and neurofibrillary tau tangles that are associated with progressive cognitive impairment and memory loss [1,2]. A large sum of data reports mitochondria malfunction, energy metabolism, and oxidative damage in AD [3,5,6,7,8]. However, the direct effect of tau pathology on mitochondria is not clearly understood and little is known about its mechanism.

Here we aim to illustrate the effect of AD-tau, on mitochondria using tau FRET biosensor cell model. FRET biosensor cell model is a quantitative, ultrasensitive and specific assay for detecting tau aggregation in-vitro. This model is derived from transducing HEK 293T cell with two separate lentivirus constructs encoding recombinant P301S-tau tagged with either cyan fluorescence protein (CFP) or yellow fluorescence protein (YFP). Introduction of potent tau seeds into these cells leads to nucleation of endogenous tau reporter proteins that produces FRET signal which can be detected through florescence microscopy and quantified using flow cytometry [9].

We treated FRET biosensor cell models with specific concentration of human AD brain derived pathological tau. After 48 hours of incubation, tau aggregation was validated on florescence/ confocal microscopy. Red mito-tracker dye, which is a molecular probe that specifically stains mitochondria was used to facilitate their visualization. We used flow cytometry to measure the combined FRET densities of both AD-tau and mitochondria, as per standard protocol.

Aggregation of AD-tau in FRET biosensor cells were successfully validated after spontaneous uptake. We found that tau aggregation reduced the mitochondrial membrane potential in these cells, analysed by flow cytometry. Co-localization of FRET signal and red mito-tracker demonstrated a probable effect on the distribution of mitochondria inside the cell.

Based on these results, we can ascertain that tau aggregation in FRET biosensor cells may affect mitochondrial membrane potential however additional investigation is needed to confirm this effect.

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THE INTERCONNECTION OF SENESCENCE AND ALPHA-SYNUCLEIN RELATED PATHOLOGIES IN NEURODEGENERATION

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Parkinson's disease (PD) is a neurodegenerative disease, affecting primarily the population over the age of 65 [1]. Prominent hallmarks of PD are accumulation of intracytoplasmic protein inclusions called Lewy bodies (LB) and progressive loss of dopaminergic neurons [2]. LB mainly consists of a protein called alpha synuclein (a-Syn), which is a natively disordered protein predominantly expressed in the brain. The duplication or triplication of SNCA (gene encoding a-Syn) locus was observed in some fami-lies with PD and its higher expression level has been suggested to play a critical role in the pathogenesis of PD [3]. Accumulation of LB leads to various detrimental effects including dysfunction of mitochon-dria, oxidative stress and synaptic dysfunction [4]. However, the precise molecular and cellular mecha-nism of a-Syn involvement in neuronal death resulting in neurodegeneration remains unclear. Some of these toxic effects are related to the process of senescence, which is a cellular mechanism resulting in the cell cycle arrest protecting the organism against the growth of severely damaged cells. Despite being an important tumour-suppression mechanism, senescence is also believed to be a major contributor to ageing and age related diseases.

Understanding the pathological changes, diagnosis, and treatment of PD is one of the pressing issues of present-day neuroscience, necessitating the development of robust disease models. In our study we cre-ated novel neuronal cell lines stably overexpressing a-Syn (wild type or its PD-related mutated variant A53T) tagged with GFP in uniform genetic background, enabling more consistent data acquisition. These newly generated cell models were then separated into three types each, based on the expression levels of a-Syn using cell sorting. We then analysed cell viability, expression levels using Western blot and qPCR and compared biological effects triggered in these cell models as the result of alterations of a-Syn levels, comparing the effects of over-expression of a-Syn among themselves and with cells not over-expressing a-Syn. We further plan to correlate the readouts with senescence markers (SA-beta-galactosi-dase staining and analysis of p16INK4a, p21Waf/Cip1, IL-6, TNFα expression).

Our study aims to enhance understanding of a-Syn related pathologies in PD and con-tribute to the es-tablishment of innovative approaches for its treatment.

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CONSTRUCTION OF CDR3-PHAGE LIBRARY FOR SELECTION OF NEUTRALIZING PEPTIDES AGAINST TICK-BORNE ENCEPHALITIS VIRUS

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Tick-borne encephalitis virus (TBEV) causes severe infections of central nervous system and more then 12 000 cases are being reported each year. Several technologies are being developed for treatment, diag-nosis and prevention of the infection. However, we still lack the effective therapeutics that could pre-vent severe and life-threating TBEV infection. We are aiming to develop of structurally constrained CDR3 peptides (Cys-CDR3-Cys) recognizing specifically protein E of the TBEV. Here, the CDR3 is de-rived from heavy chain only antibodies of llama (Lama glama). We immunized llama with recombi-nant DIII subunit of E protein. After six booster doses, mRNA from the B-lymphocytes of the llama was isolated and reverse transcribed. CDR3 region was amplified using degenerated primers. Primers were designed as 63-mer (reverse) and 35-mer (forward) with overhangs that contained restriction sites for NcoI (C/CATGG) and NotI (GC/GGCCG) restriction enzymes. The amplified product of CDR3 region was 150 bp in size. The PCR product was cloned into pSEX81 phagemid and transformed into the E. coli XL1-blue in order to generate CDR3- E. coli library. For obtaining CDR3-E. coli library, 15 electro-porations were performed to electrocompetent E. coli XL-1 blue. For the growth of clones, only one plate (17.8 cm in diameter) supported with 1% glucose and Carbenicillin was used. Clones were select-ed based on antibiotic resistance for Carbenicillin at the concentration of 1 µg/100 ml. The estimated library size was calculated as 1.1×10^{10} of clones. Clones grown on the 2xTY plates were scrapped in 50% glycerol and stored at -80 until use. A loopful of library was streaked on 2xTY-carbenicillin plate to sep-arate the clones. DNA from the clones was isolated and subjected to the sequencing using phagemid specific primers. PCR prod-ucts from 16 clones were sequenced, sequences were translated in-silico into amino acids and aligned. Based on the alignment we deduced high diversity of constructed CDR3-E. coli library 87.5%. The library was subjected to the phage packaging wherein CDR3-E.coli library was grown in 2xTY- carbenicillin till OD600 0.5, superinfected with

hyperphage (Progen, Germany) and es-caped phages were purified with NaCl-PEG precipitation. Total 1.61x1013 phages were obtained and stored in -80C until their use in biopanning against TBEV and WNV. We hypothesized that structurally constrained CDR3 selected specifically against DIII domain of protein E, which is the only binding site of flaviviruses to the host cell, could block the binding of virus to the host cell receptor and thus its cell entry.

ANIMAL MODELS OF TBI, FIRST EXPERIENCE AT NIU SAS

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Traumatic brain injury (TBI) is sustained by tens of millions of patients each year. TBI of mild severity, characterized by a transient disruption of brain function, is the most prevalent form. Understanding the underlying mechanisms and developing effective therapeutic interventions for mTBI necessitate the use of appropriate animal models that mimic the human condition. In this study, we aimed to develop a mouse model of mTBI to provide a reliable and reproducible experimental platform for investigating the pathophysiology and potential therapies.

The mouse model was established by utilizing an electromagnetic controlled cortical impact device that delivered a controlled force to the closed skull of anesthetized mice. The impact parameters, including the impactor tip size, velocity, dwell time, and depth, were carefully optimized to induce a mild injury with minimal mortality and consistent behavioural deficits. Various outcome measures, such as neuro-behavioral assessments, histological analysis, and biochemical assays, are being employed to evaluate the severity and progression of the injury.

The mouse model of mTBI in development holds great promise for advancing our understanding of the underlying mechanisms and exploring potential therapeutic interventions for this prevalent form of TBI. The model's reproducibility and similarity to the human condition make it an invaluable tool for preclin-ical studies aiming to unravel the complex pathophysiology of mTBI and evaluate the efficacy of novel treatment strategies. Ultimately, this model may contribute to the development of effective interven-tions and improve outcomes for individuals suffering from mTBI.

INVESTIGATION OF METASTABLE CONFORMATIONS OF THE NEURONAL PROTEIN TAU TO DESIGN NOVEL ANTI-AD DRUG CANDIDATES

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Unlike globular proteins with stable 3D structures, intrinsically disordered proteins exist as a highly dynamic system composed of interchanging conformations, which complicates their structural studies. These disordered proteins are often subjected to a self-aggregation process yielding insoluble protein aggregates that damage neurons, as in the case of various neurodegenerative diseases. Intracellular in-clusions of protein tau are present in the brains of Alzheimer's disease patients [1]. The aim of the re-search is to identify metastable tau conformations against which inhibitors of aggregation can be de-signed. To achieve our objectives, we combine X-ray crystallography with molecular dynamics simula-tions and molecular docking experiments [2-3]. Future plans include screening the virtual library of small molecules to select potential molecules that affect the aggregation of tau.

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MESENCHYMAL STEM CELL THERAPY FOR THE TREATMENT OF TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is a major cause of injury-related mortality and morbidity. TBI can occur due to head impacts such as sports-related injuries, accidental fall, domestic violence, automobile accidents, or blast-related injuries. Those who survive TBI may experience a range of symptoms, including cognitive and memory impairments, vision and hearing loss, movement disorders, and various psychological challenges. Based on the severity of primary impact or damage as well as the extent of secondary damage, TBIs can be categorized into mild, moderate, and severe [1-2]. The treatment of TBI induced diseases are still symptomatic and very challenging. Clinical trials for drugs aimed at restoring neuronal loss have not been successful so far. However regenerative approaches for TBI treatment using mesenchymal stem cells (MSCs) seem to be promising [3]. The success of stem cell therapy depends on optimal transplantation time point and route of administration. Also, differentiation of transplanted MSCs is considered as an important step of successful therapy. MSCs have potential to differentiate into a variety of adult cells, like chondrogenic, osteogenic, and neural progenitors [4]. Preclinical research has demonstrated that transplanting MSCs has the potential to decrease secondary neurodegeneration and neuroinflammation, stimulate neurogenesis and angiogenesis, and enhance overall functional outcomes in experimental animals.

In the project, we are focussing on the efficient preparation of mouse MSCs cultures, expansion and subsequent differentiation into nerve cells. For differentiation, we used different combinations of inducers such as β-mercaptoethanol, forskolin or IBMX. So far, we analysed the stem cells by immunofluorescence via confocal microscopy and detected the expression of tau protein, β-tubulin III and nestin. Our results indicate that the differentiation of stem cells into nerve cells is challenging and requires further experimentation.

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DOMAIN III OF ENVELOP GLYCOPROTEIN OF WEST NILE VIRUS (WNV) AFFECTS SIGNALLING EVENTS IN HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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West Nile virus (WNV) causes flu like symptoms or serious meningoencephalitis. It infects humans, horses and birds. The virus is transmitted via mosquitoes of the genus Culex [1]. The attachment of a virion is mediated by domain III (DIII) of envelope glycoprotein E which is the main antigen provoking the immune response in the host [2]. Domain III attaches to the cells of a neurovascular unit and initiates a series of events that help viral entry in to the CNS. The aim was to uncover the post-attachment events elicited in brain microvascular endothelial cells (BMEC) by DIII of WNV (strain goshawk Hungary 2004, GenBank acc. n. DQ116961). cDNA fragment encoding DIII was PCR amplified, ligated into pQE-30 plasmid and overexpressed in E.coli. Human BMEC were infected with recombinant DIII, cell mRNA was isolated and cDNA libraries for RNA-seq were prepared using QuantSeq3' mRNA-Seq Library Prep (Lexogen, Austria) and sequenced on Illumina NextSeq to a minimal depth 8 million reads per sample. STAR aligner was used to process Fastq files, aligned to reference genome (GRCh38) and generate gene counts. Differential gene expression analysis was carried out by R package edgeR. Results were validated with qRT-PCR. Analysis revealed significant alteration in expression of the genes involved in cell-extracellular matrix interactions and tight-junction integrity. Genes related to vesicle-mediated transport (clathrin-mediated endocytosis), apoptosis pro-inflammatory cytokines and chemokines, cell-adhesion molecules were also evoked. Results suggest that the virus contact to the cell surface emanates a series of events in BMEC that may help in translocation of virus across blood brain barrier.

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DEVELOPMENT OF NANOBODY-BASED NANOCARRIER SYSTEM AGAINST TICK-BORNE ENCEPHALITIS VIRUS TO OVERCOME THE BLOOD-BRAIN BARRIER

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Tick-borne encephalitis virus (TBEV), belonging to the Flaviviridae family, can cause sever encephalitis, meningitis or meningoencephalitis. In rare cases, it can cause permanent brain damage or long-term neurological sequelae [1]. TBEV enters the cells after attachment of receptor-binding domain III (DIII) of the envelope (E) glycoprotein to the cell receptor. Entry of the virions in the brain microvascular endothelial cells (hBMEC) causes CNS entry of TBEV across the blood-brain barrier (BBB). Therefore, E glycoprotein continues to be a major target in the development of a therapeutic agent [2, 3]. As the current therapy is only supportive, there is a need to develop effective anti-viral therapy. The main obstacle in the distribution of drugs to the CNS is BBB, which limits passage of drugs from blood to the brain parenchyma [4]. In this study, nanobodies (NBs) were developed against the DIII of E glycoprotein of TBEV to block attachment of virus to hBMECs. NBs were generated from blood mononuclear cells of llama immunized with recombinant DIII (rDIII) of TBEV. A phage library presenting repertoires of NBs was synthesized and panned against rDIII. The soluble NBs were produced in E. coli SHuffle expression system and shortlisted on the basis of strong affinity to rDIII, and the ability to block binding of rDIII to hBMEC (Bio-layer interferometry, ELISA). NBTA3 and NBTT9 targeting rDIII was used to neutralize TBEV in plaque reduction neutralization test (PRNT) with EC50 of 2.5 µg/ml (333 nM) and were conjugated with nanosystem containing polyamidoamine (PAMAM) dendrimer of 4th generation, decorated with angiopep-2 (CNS-homing peptide) and loaded with Cy5.5 dye (nanobody-based dendrimer nanosystem, nDDs). NBTT9-nDDs demonstrated a 14% ability to cross the in vitro BBB model, whereas non-conjugated NBTT9

did not. Moreover, NBTT9-nDDs neutralized TBEV in PRNT with EC50 of 0.17 μg (22.7 nM). Finally, cytotoxicity and hemocompatibility of NBTT9-nDDs were tested in vitro resulting in 100% hBMEC viability and 0% hemolysis in the presence of 66.7 μM of nanosystem. The neutralizing activity of NBTT9-nDDs after crossing in vitro BBB model will be tested. NBTT9-nDDs seems to be a promising therapeutic for the treatment of TBEV induced neuropathogenesis.

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PRODUCTION OF ANTI-DENGUE APTAMERS USING DENGUE VIRUS LIKE PARTICLES

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Dengue is considered as a Neglected tropical disease (NTD) by WHO causing periodic endemics. In recent years, there has been a rise in cases of dengue haemorrhagic fever (DHF) implying that virus is developing neurotropic affinity. There is no approved therapeutic agent for human use, but for prevention, CYD-TDV Dengavaxia (Sanofi Pasteur Inc.), a live, tetravalent, FDA-approved vaccine for people with a history of infection, is available. Other potential candidates are Takeda (TAK-003) and TV003/TV005 developed by National Institute of Allergy and Infectious Diseases. The Dengue virus belongs to flaviviridae family, and has a positive, single stranded and enveloped RNA. The molecular structure is composed of three structural and seven nonstructural proteins [1]. The Envelope protein consists of three ectodomains viz., domain I, II and III. The Domain III of E protein facilitates the binding of the DENV to different receptors of cells [2]. Hence there arises the need to produce a blocking agent that would bind to the of E protein and thereby inhibit the binding of the virus to the cells. Aptamers can be considered to be a suitable candidate for therapeutic purposes due to its ability to accurately bind to the target, smaller size, zero cross reactions, low toxicity and low cost production [3]. The first aptamer produced against dengue was able to bind to DENV-2 envelop protein domain III (D3) and also neutralize the infections caused by all four serotypes of DENVs [4]. Further, first RNA based aptamer was produced against the 5´-UTR (untranslated regions) coding for polyprotein, which lead to structural changes of the proteins [5]. In the current study we have successively produced single stranded aptamer library that can specifically bind to Dengue virus serotype 2 VLP (Native antigen) using Systematic Evolution of Ligands by Exponential Enrichment (SELEX). A total of eight rounds of SELEX were performed and the binding affinity of the aptamers was analysed using digoxigenin (DIG) ELISA. The pool of specific aptamers were cloned, and after colony qPCR, the clones were categorised according to the data obtained after melt curve analysis. Ten groups of potential candidates were made with similar melting temperatures. These aptamers can be used

as a therapeutic agent in a nano drug delivery system (nDDS) by conjugating it with nanocarriers like dendrimers which would help it cross the blood-brain barrier and bind specifically to domain III and thereby inhibit binding of the virus to the cells.

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DENDRIMER AS AN ANTIMICROBIAL AGENT AGAINST *NEISSERIA MENINGITIDIS*

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Bacterial meningitis is a serious neurological disease that often results in death. Neisseria meningitidis serogroup B (NM) belongs to meningitis causing bacteria. One of the novel therapeutic approaches is the use of antimicrobial nano-formulations that can cross the blood-brain barrier (BBB) and be effective at the site of infection [1, 2]. The aim of this study was to test antimicrobial activity of amphiphilic dendrimers against NM in comparison to the routinely used ciprofloxacin (Cip). NM isolated from patient diagnosed with meningitis at The University Hospital Olomouc, CR, was cultured in brain heart infusion broth at 37 °C, 5% CO_2 up to mid-exponential phase. A total of 25 dendrimer formulations were first tested for their cytotoxicity and nontoxic dendrimers were subjected to find the minimum inhibitory concentration (MIC) against NM. Likewise, the MIC of Cip was also determined. Bacterial culture was incubated with different concentration of two-fold serially diluted drugs. To measure the effective time to kill NM for each drug, bacterial culture was incubated with MIC obtained. In control wells, the drugs were replaced with dH_2O. During both experiments, samples were incubated at 37 °C, 5% CO_2 for desired time and subsequently plated on chocolate agar plates containing 5% of sheep blood to count the recovered colony forming units. Among noncytotoxic dendrimers tested against NM, we found that DDOle-8TA and MBola-8T had MIC 50 µM, whereas, DDC18-8TA had MIC 6.25 µM only. The MIC of Cip was 37.5 µM. The effective concentration of DDC18-8TA against NM is six time less compared to Cip. Their effectivity to kill half of living bacteria is 3 and 5 hours, respectively. Despite the fact, that ciprofloxacin is potent against NM in culture broth, its usage the patients with meningitis is hampered by its ineffective crossing through the BBB [1]. Some of the dendrimers, the nanocarriers, possess inherent ability to cross the BBB. This property could be exploited further to develop a dendrimer based nanocarrier system to transport antibiotics across the BBB.

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SYNTHESIS OF STRUCTURALLY CONSTRAINED CDR3 PEPTIDES AGAINST SARS-COV-2

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused the outbreak of a global pandemic with the first breakout reported in the Hubei province of Wuhan in late 2019 [1]. Although the clinical manifestation of the COVID-19 disease caused by the virus predominantly involves the res-piratory system, recent reports suggest that the pathology of the virus may extend beyond it to involve other organs including the brain [1]. Peptides derived from the CDR3 region of the llama VHH, specifi-cally selected against protein S of SARS-CoV-2 could be potential viral neutralizing molecules. Due to the small size of CDR3-derived peptides, stability, and specific interaction with epitopes, these mole-cules represent a promising alternative in the fight against SARS-CoV-2 [2]. In the experimental proce-dure, first, the blood was collected from Llama glama immunized with protein S of SARS-CoV-2. mRNA was isolated and reverse transcribed into cDNA. cDNA was amplified by SfiI (F) and SfiI (R) primers and its total size was 120bp. Primers were designed prior to amplifica-tion of CDR3 DNA region of the nanobody sequence. They contained degenerated bases to obtain great diversity of sequences and furthermore overlapping regions which were not aligned with the template but enabled to create com-plementary overhangs for cohesive ends ligation. In overhang sequences of both primers were restriction sites for SfiII restriction endonuclease (GGCCNNNN/NGGCC). Amplicons were digested with SfiI, cloned into pJB12 phagemid and transformed into E. coli XL1-blue to produce a CDR3-E. coli library. For obtaining CDR3-E. coli library, 14 electro-porations were performed to electrocompetent E. coli XL-1 blue. For the growth of clones, 3 plates (17.8 cm in diameter) with 1% glucose and tetracycline were used. Library was super infected with VSCM13 Interference Resistant Helper Phage to es-cape the pack-aged phage particles. To select the CDR3 specific against protein S, we performed a total of 5 rounds of biopanning on VLP of SARS-CoV-2. After each round, we got more specific phages with CDR3. Inter-action of phages isolated from the last round of biopanning to the protein S was confirmed by ELISA. DNA from the

pool of the phages after last round was subjected for DNA isolation, CDR3 fragments were amplified with the same primers described above, however the flaking regions had BamHI and SalI sites. Amplicons were digested and ligated into UA-mCherry-GFP plasmid and electroporated. E.coli Shuffle were transformed with the recombinant plasmid and 96 clones were selected randomly to check their ability to produce CDR3. Each CDR3 clone will be purified and tested for its binding affinity to protein S. The best binder will be shortlisted and used in the development of the nano-carrier based drug against SARS-CoV-2.

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