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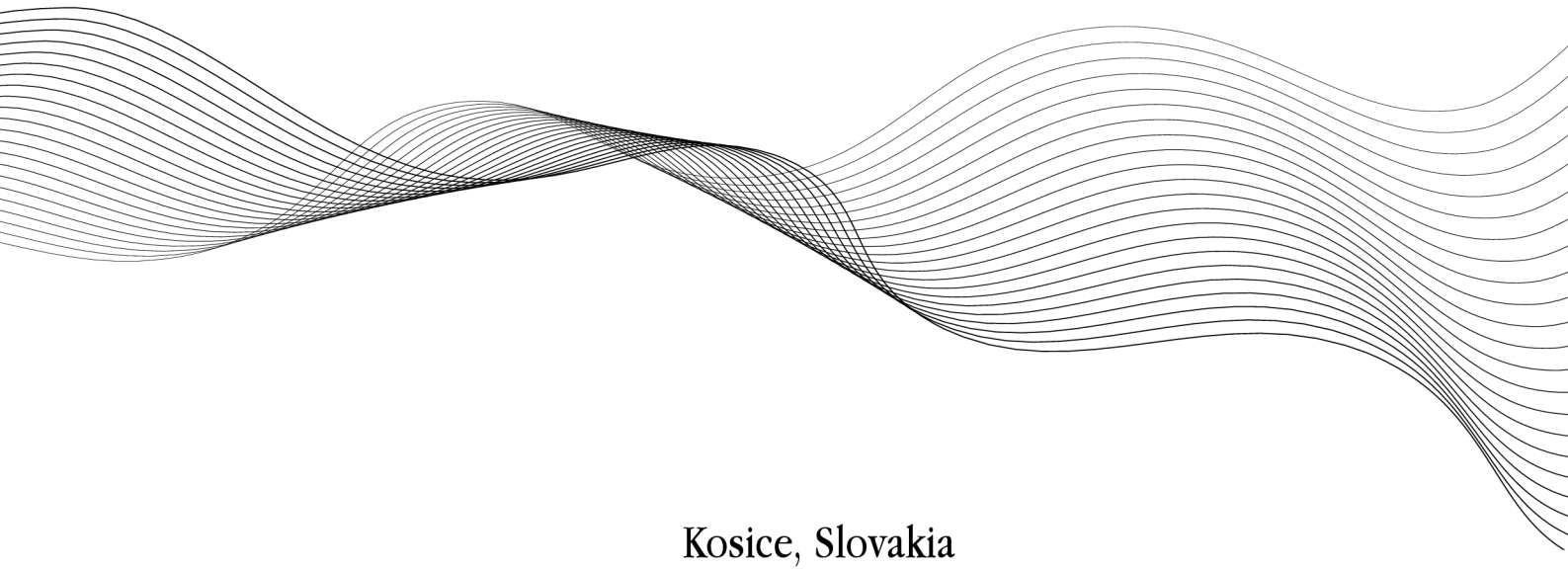

Kosice, Slovakia

June 2 – 5

2024

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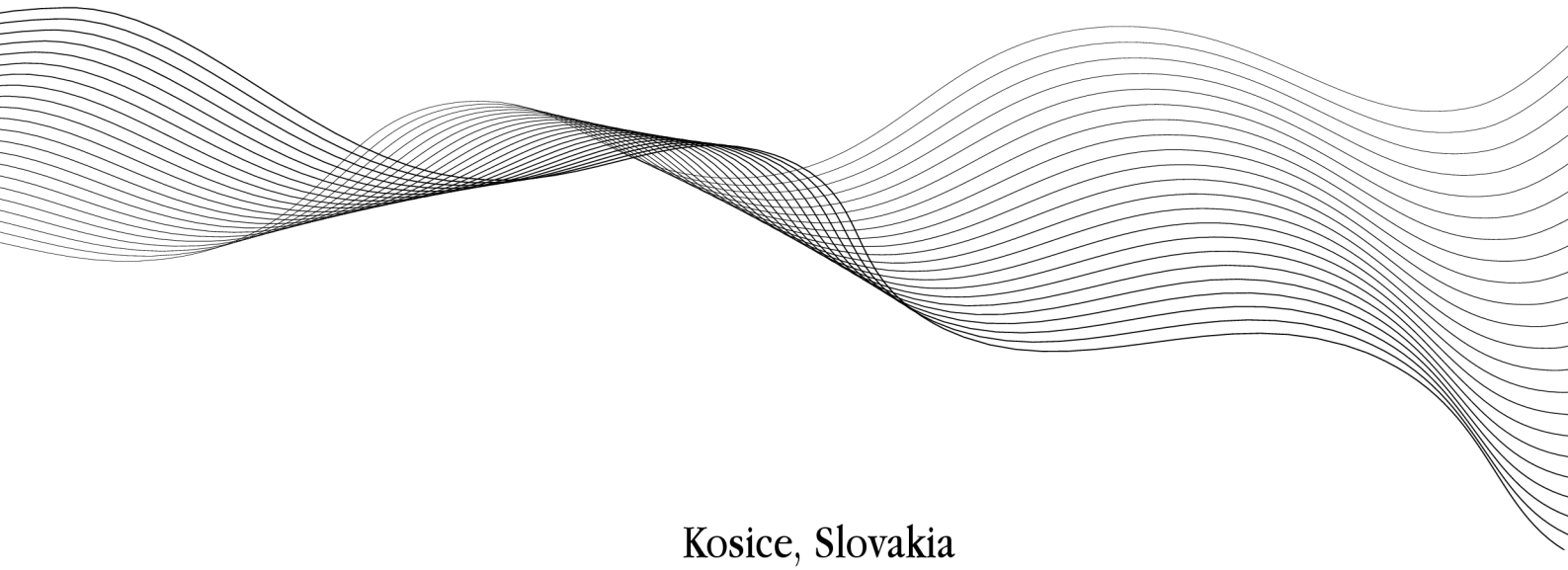

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9th International Symposium on Experimental and Clinical Neurobiology

Program and Abstract Book

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Symposium Venue

Catholic University, Hlavna 89, Kosice

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General schedule of events

Sunday, June 2, 2024

16:00 –19:00	Registration, Symposium venue, Catholic University, Hlavna 89, Kosice

Monday, June 3, 2024

08:00 – 15:00	Registration – Symposium venue
08:00 – 08:50	Uploading presentations and posters installations
09:00 – 09:15	Opening of the Symposium
09:15 – 10:00	Plenary lecture
10:00 – 10:55	CNS injury - mechanisms and neuroprotection I
11:00 – 11:20	Coffee break, posters
11:20 – 12:25	CNS injury - mechanisms and neuroprotection II
12:30 – 13:30	Lunch
13:30 – 14:00	Poster session I
14:00 – 14:55	CNS injury - mechanisms and neuroprotection III
15:00 – 15:20	Coffee break, posters
15:20 – 16:35	Varia I
17:00 – 20:00	Get together party

Tuesday, June 4, 2024

08:00 – 15:00	Registration – Symposium venue
08:00 – 08:50	Uploading presentations and posters installations
09:00 – 09:45	IBRO lecture
09:50 – 10:50	CNS injury - mechanisms and neuroprotection IV
10:50 – 11:10	Coffee break, posters
11:10 – 12:25	Neurodegenerative Diseases & Clinical Neuroscience I
12:30 - 13:30	Lunch
13:30 – 14:00	Poster session II
14:00 – 14:45	Neuroregeneration – cell based therapy, neurogenesis I
15:00 – 20:00	Trip and wine tasting in Tokaj region

Wednesday, June 5, 2024

08:00 – 10:00	Registration – Symposium venue
08:00 – 08:50	Uploading presentations and posters installations
09:00 – 10:25	Varia II
10:30 – 10:50	Coffee break
10:50 – 12:05	Varia III
12:05 – 12:30	Closing of the Symposium
12:30 – 14:00	Lunch

Scientific program

Monday, June 3, 2024

09:15 – 10:00 **Plenary lecture**
iNets - novel human neural networks to study neurodegenerative diseases.
Hruska-Plochan M.

CNS injury - mechanisms and neuroprotection I
Chairperson: Motlík J.

10:00 – 10:25 Processing of nociceptive peripheral input in spinal cord networks.
Krotov V., Agashkov K., Blashchak I., Andrianov Y., Romanenko S., Halaidych O., Koroid K., Keyes A.L., Safronov B., Voitenko N., Usachev Y.M., Belan P.

10:25 – 10:40 3D printed scaffolds for repair of injured spinal cord and peripheral nerves.
Medvediev V., Grebenyuk S., Dobropolska Y., Sheremet Y., Abdalla I., Ustymenko V., Bomikhov O., Pivneva T., Ranga A., Belan P., Voitenko N.

10:40 – 10:55 Application of tubular conduit in transected rat tail nerve.
Blaško J., Mojžišová M., Székiová E., Michalová Z., Vanický I.

CNS injury - mechanisms and neuroprotection II
Chairperson: Strnadel J.

11:20 – 11:45 Mechanism of selective neurodegeneration after global brain ischemia. Proteasomal versus endoplasmic reticulum stress.
Ziakova K., Kovalska M., Pilchova I., Dibdiakova K., Brodnanova M., Pokusa M., Kalenska D., Racay P.

11:45 – 12:00 Blood cells-derived secretome- a novel approach for the treatment of ischemic stroke outcomes.
Bonova P., Koncekova J., Kotorova K., Nemethova M., Bona M., Gottlieb M.

12:00 – 12:25 Multiple roles for Angiogenin as a new clinical target for stroke. ONLINE
Rosell A.

12:30 – 13:30 **Lunch**

CNS injury - mechanisms and neuroprotection III
Chairperson: Pavel J.

14:00 – 14:25 Brain myelin as an energy source. ONLINE
Matute C.

14:25 – 14:40 Evaluation of the analgesic potential of recombinant GABAergic hiPSCs and their exosomes in a model of spinal cord injury induced pain. ONLINE
Jergova S., Behnaz Rahimi B., Pressman Y., Tierney L., Sagen J.

14:40 – 14:55 Behavioral, morphological, and synaptic changes in valproate-induced autism rat model.
Bakoš J., László K., Vörös D., Havránek T., Mihalj D., Kupková K., Bogyová E., Bačová Z.

Varia I

Chairperson: Voitenko N.

15:20 – 15:35 Mirtazapine intake during pregnancy has only moderate effect on hippocampal excitability of the offspring.
Lacinova L., Dubiel-Hoppanova L., Idunkova A., Bukatova S., Ondacova K., Tomko M., Jurkovicova-Tarabova B., Dubovicky M.

15:35 – 15:50 Carotid endarterectomy – potential induction of ischemic tolerance in clinical praxis.
Sihotsky, Mucha, Kopolovets, Furman, Nemethova, Virag

15:50 – 16:05 Clinical use of the phenomenon of ischemic tolerance.
Burda R., Némethova M., Burda J.

16:05 – 16:20 Unlike in other vertebrates, majority of cerebrospinal fluid-contacting neurons in the spinal cord of C56Bl/6N mice is present in ectopic position.
Košuth J., Tonelli Gombalová Z., Alexovič Matiašová A., Zrubáková J., Žežula I., Daxnerová Z., Ševc J.

16:20 – 16:35 Emission of 50-kHz ultrasonic vocalizations in hemiparkinsonian rats as a new preclinical approach to study the affective properties of drugs used in the dopamine replacement therapy of Parkinson's disease.
Serra M., Marongiu J., Simola N., Costa G.

18:00 – 20:00 **Get together party**

Posters I – Monday, June 3, 2024

P-01 Selected genetic indicators of induced brain stroke ischemic tolerance found in human blood: A quantitative analysis.
Furman M., Sihotsky V., Virag M., Kopolovets I., Nemethova M., Mucha R.

P-02 The impact of traumatic spinal cord injury on the expression of Angiotensin II receptors within the hypothalamus-pituitary-adrenal axis.
Hvozdikova E., Liptakova V., Snopkova J., Kellerova E., Pavel J.

P-03 Body weight-supported treadmill training in spinal cord injured rats.
Ihnátová L., Magurová M., Kisucka A., Kuruc T., Ileninová M., Kucharová K., Lukačová N., Gálik J.

P-04 The tryptophan-kynurenine pathway- therapeutic strategy for neuroprotection in tauopathies.
Khiratkar K., Majerova P., Kovac A.

P-05 The impact of subpial administration of Chondroitinase ABC on glial scar components following SCI.
Kisucká A., Kiss Bimbová K., Bačová M., Ileninová M., Kuruc T., Kuchárová K., Ihnatová L., Lukáčová N.

P-06 Remote ischemic conditioning modulates the inflammation after stroke in the rat model of hyper-inflammation.
Končeková J., Kotorová K., Némethová M., Bonová P.

P-07 Blood cell-derived secretome as an alternative stroke treatment approach for obese individuals.
Kotorová K., Konceková J., Gottlieb M., Bonová P.

P-08 Aerobic exercise-driven brain resilience: Insights into hippocampal alterations of obese rats.
Kuruc T., Kuchárová K., Kisucká A., Ileninová M., Ihnátová L., Kiss Bimbová K., Magurová M., Gálik J., Lukáčová N.

P-09 Neuroprotective and anti-inflammatory effects of neuroactive steroids in model of perinatal focal cerebral ischemia.
Kútna V.,Holásek M.,Kudová E.,Chodounská H.,Druga R., Tsenov G.

P-10 The effect of Angiotensin receptor type 2 stimulation on neuroregeneration after severe spinal cord injury.
Liptakova V., Snopkova J., Hvozdikova E., Pavel J.

P-11 Siponimod shows greater efficacy than Methylprednisolone and Atorvastatin in reducing neuroinflammation and promoting recovery after spinal cord injury.
Lukáčová N., Kiss Bimbová K., Kisucká A., Bačová M., Ileninová M., Kuruc T., Kuchárová K., Ihnatová L., Magurová M., Gálik J.

P-12 Exploring combination therapy with epidural stimulation and atorvastatin for spinal cord injury recovery in rat model.
Magurova M., Bacova M., Papcunova S., Galik J.

P-13 The effect of siponimod on alpha-synuclein expression and inflammatory markers in a spinal cord injury model.
Motyl J., Wencel P., Kisucka A., Czubowicz K., Lukacova N., Strosznajder R.

P-14 Proteomic analysis of signaling pathways in the rat hippocampus after delayed remote ischemic postconditioning.
Némethová M., Talian I., Tkáčiková S., Mucha R., Končeková J., Kotorová K., Bonová P.

P-15 The stimulation of AT2 receptors can promote an angiogenic response after severe spinal cord trauma.
Snopkova J., Liptakova V., Hvozdikova E., Pavel J.

P-16 The role of sphingosine-1-phosphate receptor modulator (Siponimod) in spinal cord injury.
Wencel P., Kisucka A., Motyl J., Lukacova N., Strosznajder R.

Tuesday, June 4, 2024

09:00 – 09:45 **IBRO lecture**
Advancing spinal cord injury repair through multidisciplinary therapeutic approaches.
Silva N.

CNS injury - mechanisms and neuroprotection IV
Chairperson: Hruska-Plochan M.

09:50 – 10:05 Is Angiotensin II receptor type 2 involved in potentiated intrinsic regenerative ability in injured spinal cord?
Pavel J., Liptakova V., Hatalova E., Snopkova J.

10:05 – 10:20 Neonatal nervous tissue exhibits superior reparative potential compared to the mature nervous tissue after minimal spinal cord injury.
Ševc J., Mochnacký F., Košuth J., Alexovič Matiašová A., Slovinská L., Blaško J., Bukhun I., Holota R., Tomori Z., Daxnerová Z.

10:20 – 10:35 Neurotrophic factor expression in different microenvironments six weeks after thoracic spinal cord injury and carnosine treatment.
Kuchárová K., Kuruc T., Ihnátová L., Gálik J., Magurová M., Ileninová M., Kisucká A., Lukáčová N.

10:35 – 10:50 Modulation of the gut-spinal cord axis by probiotic treatment in the Th9 compression model.
Ileninová M., Kiss Bimbová K., Kisucká A., Bačová M., Mudroňová D., Kuruc T., Gálik J., Kuchárová K., Ihnátová L., Lukáčová N.

Neurodegenerative Diseases & Clinical Neuroscience I
Chairperson: Jezova D.

11:10 – 11:35 Induced pluripotency and its potential in modeling of oncologic and neurodegenerative diseases.
Strnadel J.

11:35 – 12:00 Phenotypical, genotypical and pathological characterization of the moonwalker mouse, a model of ataxia.
Sekerkova G., Kilic S., Cheng Y.H., Fredrick N., Osmani A., Kim H., Opal P., Martina M.

12:00 – 12:25 Detrimental role of hyperhomocysteinemia in the ischemic/reperfusion conditions.
Lehotsky J, Baranovicova E, Hnilicova P, Kalenska D, Kovalska M, Kaplan P, Tatarkova Z

12:30 – 13:30 **Lunch**

Neuroregeneration – cell based therapy, neurogenesis I
Chairperson: Lehotsky J.

14:00 – 14:15 Transgenic minipig models of the serious eye diseases.
Motlik J.

14:15 – 14:30 Mesenchymal stem cells conditioned medium affects neurons and glial cells differently depending on the time of conditioning.
Székiová E., Blaško J., Michalová Z., Vanický I.

14:30 – 14:45 Mature neurons of the olfactory neurogenic region are connected with the striatum.
Fabianová K., Martončíková M., Vanický I.,Blaško J., Popovičová A.,Raček A. Račeková E.

15:00 – 20:00 **Trip and wine tasting in Tokaj region**

Wednesday, June 5, 2024

Varia II
Chairperson: Bakos J.

09:00 – 09:25 Significance of animal models of mental disorders: experimental and clinical point of view.
Jezova D., Hlavacova N., Izakova L.

09:25 – 09:40 Inhibition of aldosterone synthesis during the stress-hyporesponsive period results in negative neurobiological effects in juvenile rats.
Hlavacova N., Hrivikova K., Graban J., Karailievova L., Jezova D.

9:40 – 9:55 Ultrastructural insight on the arrangement of ependymal cells, vasculature and fractone bulbs in the central canal lining of rat spinal cord.
Alexovič Matiašová A., Týč J., Herranz-Pérez V., Košuth J., Malčický L., Vancová M., Nebesářová J., Daxnerová Z., García-Verdugo J.M., Ševc J.

9:55 – 10:10 In search for an optimal tool for detection of apoptosis in nervous tissue: Cleaved caspase-3 is present in the majority of glial cells in the intact rat spinal cord during postnatal life.
Holota R., Dečmanová V., Alexovič Matiašová A., Košuth J., Slovinská L., Pačut L., Tomori Z., Daxnerová Z., Ševc J.

10:10 – 10:25 Persistent olfactory dysfunction after COVID-19: the results of remote olfactory testing and data collection in Slovakia.
Martončíková M., Doležal P., Fabianová K., Karhánek M., Gálik J., Raček A., Popovičová A., Račeková E.

Varia III
Chairperson: Kucharova K.

10:50 – 11:05 Bridging the gap: P2X7 receptor as a molecular link between α-synuclein toxicity, Parkin dysfunction, and mitochondrial Impairment.
Wilkaniec A., Olech-Kochańczyk G, Babiec L., Czapski G.A., Lenkiewicz A.M. Adamczyk A.

11:05 – 11:20 Current advances in thrombolytic research - how to break the translational block in preclinical research.
Hložková J., Scheer P., Ondruš J., Goliášová S., Aksu Davut A., Brhelová E., Thalerová S., Biskupič J., Kuchynka M., Mikulík R.

11:20 – 11:35 Genes expression altered in activation of ischemic tolerance through carotid endarterectomy in human blood.
Mucha R., Furman M., Sihotsky V., Kopolovets I., Virag M., Nemethova M.

11:35 – 11:50 Genetic forms of Parkinson's Disease in Central Europe
Ostrozovicova M, Tamas G, Dusek P, Grofik M, Han V, Holly P, Jech R, Kalinova K, Klivenyi P, Kovacs N, Kulcsarova K, Kurca E, Lackova A, Lee H, Lewis P, Magocova V, Marekova M, Murphy D, Necpal J, Pinter D, Rabajdova M, Ruzicka E, Serranova T, Smilowska K, Soos K, Straka I, Svorenova T, Valkovic P, Zarubova K, Gdovinova Z, Houlden H, Rizig M, Skorvanek M.

11:50 – 12:05 Heart rate variability in the assessment of autonomic dysfunction in idiopathic REM-sleep behavior disorder.
Ventosa J.

12:05 – 12:30 Closing symposium

12:30 – 14:00 **Lunch**

Posters II – Tuesday, June 4, 2024

P-01 Oxidative stress, neuroinflammation and astrogliosis in an α-synuclein seeding/spreading mouse model of Parkinson's disease.
Adamczyk A., Ruiz-Ortega E.,Olech-Kochańczyk G., Czapski A., Cieślik M., Gawinek E., Wilkaniec A.

P-02 High-purity extracelullar vesicles used as a brain biopsy in neurodiagnostic.
Bello O.D., Rivera L., Bustos D.M., Muñoz E.M., Filipčík P., Škrabana R.

P-03 Diaschisis-related changes in main excitatory neurotransmitter glutamate in rat brain following the MCAO.
Bona M., Hvizdosova N., Kollarova P., Koncekova J., Kotorova K., Bonova P.

P-04 Studying unconstrained behavior of mice in a labyrinth to explore cognitive deficits.
Gášpárová L., Ercsényiová S., Trudičová D., Štefan J.J., Hromádka T.

P-05 Negative perception of a stressful situation is not associated with salivary concentrations of the stress hormone cortisol in toddlers.
Vancova A., Romanova Z., Jezova D.

P-06 PPAR-α synthetic ligands and their effect on the level of mRNA expression related to redox state.and mitochondria proteins in different parts of the brain in an animal model of Alzheimer's disease
Żulińska S., Czubowicz K, Strosznajder J.B.

P-07 Cell therapy for traumatic brain injury, current status and future plans – involvement of 14-3-3ζ.
Durgala A., Rivera L., Škrabanová M., Čente M., Škrabana R., Filipčík P.

P-08 Behavioral and functional consequences of maternal separation of various duration in rats.
Raček A., Žideková M., Martončíková M., Fabianová K., Račeková E.

P-09 A model for studying long distance axonal regeneration in the rat.
Vanický I.

P-10 Effect of early chronic stress on proliferation and migration of neuroblasts in the rat rostral migratory stream.
Žideková M., Martončíková M., Fabianová K., Raček A., Račeková E.

P-11 Are aged microglia capable of surprising? Responsiveness of microglial cells within the pineal gland during aging.
Freites C.L., Avila M., Muñoz E.M.

P-12 Comparative study of imaging methods and therapy results in diseases of the spine in dogs at the Veterinary University Hospital in Kosice.
Kuricova M., Zeleznik P., Torok M., Liptak T., Fuchs J.

P-13 The analysis of apoptotic and non-apoptotic roles of caspase-3 in cells of the rat spinal cord in the postnatal period.
Pačut L., Holota R., Košuth J., Matiašová A.A., Daxnerová Z., Ševc J.

P-14 How to evaluate the efficacy of thrombolysis in vivo.
Scheer P., Hložková J., Aksu Davut A., Brhelová E., Kuchynka M., Mikulík R.

P-15 Evidence that PECAM-1 is a component of Reissner´s fiber produced by cells of the subcommisural organ in rats, but not in mice.
Malčický L., Alexovič Matiašová A., Barčák D., Košuth J., Daxnerová Z., Ševc J.

Oxidative stress, neuroinflammation and astrogliosis in an α-synuclein seeding/spreading mouse model of Parkinson's disease

Adamczyk A., Ruiz-Ortega E., Olech-Kochańczyk G., Czapski G.A., Cieślik M., Gawinek E., Wilkaniec A.

Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

A key pathological process in Parkinson's disease (PD) is the oligomerization/aggregation of a presynaptic protein alpha-synuclein (α-syn) and its spreading through the brain. However, the exact mechanisms of seeding, cell-to-cell transmission and neurotoxicity of α-syn are still not fully understood. In the present study, we used an α-synuclein seeding/spreading model of PD induced by striatal injection of oligomeric form of α-syn. C57/BL6 mice received bilateral intrastriatal administration of endotoxin-tested recombinant oligomeric murine α-syn (5μg of protein/5μl/side) and were euthanized at 3 hours, 7, 14, 30, 90 or 180 days post-injection. We performed quantitative analysis of α-syn inclusions, dopaminergic cells degeneration, measured free radicals level and generated proinflammatory gene expression profiles in different brain regions at different timepoints after disease induction. A significant decrease in striatal dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels were observed at early time-points after α-syn injection along with the presence of intraneuronal inclusions containing misfolded α-syn. Moreover, we demonstrated that intrastriatal α-syn administration contributes to free radical generation and neuroinflammation in the striatum and in the midbrain. We observed a significant time-dependent changes in mRNA levels of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6 in both brain structures. We also indicated that microglia and/or astrocyte activation is region-dependent in this α-syn mouse model of PD. Our observations indicate that oligomeric α-syn could be the major driver in the early phases of PD-like neurodegeneration. Oxidative stress and neuroinflammation, could play an important role in this process.

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Behavioral, morphological, and synaptic changes in valproate-induced autism rat model

[1,2]Bakoš J., [3,4]László K., [3,4]Vörös D., [1,2]Havránek T., [1]Mihalj D., [1]Kupková K., [1]Bogyová E., [1]Bačová Z.

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Although the role of dopaminergic pathways in connection with motor functions, reward processing and motivation is extensively studied, their developmental aspects are less known. Moreover, dopaminergic pathway dysfunction is implicated in the etiology of autism spectrum disorder (ASD). Prenatal valproate (VPA) administration to mothers can lead to autistic symptoms in offspring, thus it is widely experimentally used to understand ASD and investigate the disorder's neurobiological basis. The aim of the present studies was to assess 1) the shape and growth of primary neurons isolated from dopaminergic brain areas 2) the expression of synaptic proteins in dopaminergic brain areas on postnatal day 5 and 3) early social communication behavior using ultrasound vocalization test of control and prenatally VPA-treated rats. Dams received a single intraperitoneal injection of VPA or saline, thereafter, on first postnatal day, primary neurons were isolated for morphology evaluation, or on postnatal day 5, behavioral tests were performed followed by tissue sample collection. A significantly lower neurite branching of neurons and a shorter length of the longest neurite were observed in neurons isolated from the tegmentum and striatum of rats prenatally affected by VPA administration compared to the control group. Cortical neurons isolated from prenatally VPA-treated animals showed significantly less branching, but no statistically significant differences were observed in the longest neurites. On postnatal day 5, a significant decrease in synaptic protein SNAP25 and chemokine fractalkine expression was observed in the striatum of VPA-treated females, but not in males. These findings suggest that potentially autistic behavioral changes in the VPA-induced animal model may relate to both changes in dopaminergic neuron morphology and alterations in synaptic proteins and chemoattractants within projection brain areas such as the striatum. Some findings also indicate sex differences, which require further investigation.

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High-purity extracellular vesicles used as a brain biopsy in neurodiagnostics

[1]Bello O.D., [1]Rivera L., [1]Bustos D.M., [1]Muñoz E.M., [2]Filipčík P., [2]Škrabana R.

[1]IHEM-CONICET, Universidad Nacional de Cuyo, Mendoza, Argentina
[2]Neuroimunologický ústav SAV, Bratislava, Slovenská republika

Neurodegenerative diseases, such as Alzheimer's and Parkinson's, are characterized by the progressive accumulation and spread of mutated or wild type misfolded proteins, which result in neurodegeneration and functional decline. Recent advances suggest that these proteins can propagate pathogenicity by transferring via extracellular vesicles (EVs) that cross cell boundaries. Our research focuses on the role of EVs, derived from microglial cells, as carriers of these misfolded proteins, potentially serving as a "liquid biopsy" for neurodegenerative conditions.

We have developed an approach using immortalized murine microglial BV2 cells exposed to tau protein fibrils (dGAE T40) to simulate the cell environment of a proteinopathy. Conditioned media from these cells undergo gradient centrifugation and flotation assays, yielding high-purity EVs. Preliminary results demonstrate the transfer of misfolded proteins through EVs. To evaluate the disease-propagating capacity, HEK293T sensor cells, which have been engineered to report the nucleation and aggregation activity of pathological proteins, are incubated with EVs loaded with dGAE T40 fibrils.

Our results hold significant promise for clinical applications, offering a less invasive alternative with the potential for early diagnosis through blood-based biomarkers of neurodegenerative and other brain diseases. Furthermore, understanding the dynamics of EV-mediated protein transfer could illuminate new therapeutic targets to disrupt the pathological progression of neurodegenerative diseases.

Acknowledgement: This work was supported by Grant number 873127 InterTAU (Marie Sklodowska-Curie Action Research and Innovation Staff Exchange Grant Agreement).

Keywords: extracellular vesicles, proteinopathies, liquid biopsy, tau protein

Application of tubular conduit in transected rat tail nerve

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Peripheral nerve injury and subsequent limitation/loss of function has long been an unsatisfactorily resolved medical issue, given the nature of the nervous system. Among the most severe forms of nerve damage is transection (neurotmesis), which results in the complete disruption of nerve continuity and, in some cases, the loss of entire nerve segments, complicating following surgical intervention. In this context, tubular conduits appear as a potentially suitable tool for bridging interrupted nerve stumps, as they can provide not only physical support but also a conducive environment for regenerative processes. In our experiment, we performed a transection on our established model of peripheral nerve damage—the rat tail nerve—and subsequently applied a tubular conduit that connected the proximal and distal nerve stumps, with the stumps placed at various distances within the conduit. Quantification of regenerated axons after the survival period showed that the tail nerve could grow through a gap of up to 5 mm between the stumps in the tubular conduit we used, but it could not bridge a 10 mm gap. These results lay the groundwork for our further experiments.

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Diaschisis-related changes in main excitatory neurotransmitter glutamate in rat brain following the MCAO.

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Middle cerebral artery occlusion is the main cause of ischemic stroke in the human population. There are lots of metabolic, biochemical and histological changes in ischemia-affected tissue but it influences also tissue distant from a stroke. This phenomenon is called "Diaschisis" and originally was characterized in 1902 by Monakow as a sudden change of function in a portion of the brain connected to a distant, but damaged, brain area. In the recent past, diaschisis includes all changes in regions distant from impacted tissue. According to types of fibres, 3 main types of diaschisis are defined. Cerebro-spinal, cortico-commissural and associative, all explained by several processes, i.e. deafferentation, oedema and last but not least spreading depression (SD). SD represents a wave of electrophysiological hyperactivity followed by a wave of inhibition and causes excessive and abnormal excretion of neurotransmitters in non-affected portions of the brain, connected to the infarcted area via axons. As glutamate is considered the main excitatory neurotransmitter in the vertebrate brain, we chose it for our analysis to evaluate the ratio of spreading depression in rat brain during infarct maturation (up to 7 days). In the present work, we measured the glutamate concentration in homogenate of 9 different parts of rat brain. While in parts that are directly affected by ischemia (core and penumbra), the glutamate increase is caused by glial disability to convert glutamate to glutamine, in unaffected parts of the ipsilateral hemisphere and the contralateral hemisphere it is most likely caused by the SD effect. In the ipsilateral hemisphere, the most noticeable changes distally from the ictus were detected in in prefrontal (decrease compared to control) and occipital cortex (increase compared to control) during the whole time of infarct maturation. Minor changes in other non-affected structures of the ipsilateral hemisphere (cerebellum, pons and hypothalamus) were monitored as well. The contralateral hemisphere shows a similar pattern of glutamate distribution changes in the prefrontal and occipital cortex. Interestingly, decreasing concentration in glutamate of the majority of structures 7 days following infarction was confirmed. Presented results show infarct maturation changes in glutamate concentration as a manifestation of diaschisis in all structures of the brain regardless of the type of axonal connection (cerebro-spinalis, cortico-commissural and associative).

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Blood cells-derived secretome- a novel approach for the treatment of ischemic stroke outcomes

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The current conception of stroke patient treatment is based on the fastest start of the therapy. Ischemic events, the most common type of stroke, are treated by clot-dissolving drugs, or by direct removal of the clot by thrombectomy. Nevertheless, only a low percentage of the affected meet the conditions for those therapies according to ASA.
Remote ischemic conditioning (RIC) has recently become an attractive approach for improving or replacing current stroke therapies in preclinical and clinical trials. RIC represents short, repeated ischemia of a distant tissue, organ, or limb, capable of inducing tolerant phenotype, i.e., stimulating endogenous mechanisms of tissue protection against lethal ischemia. Our previous research confirmed the essential role of blood circulation in RIC-mediated neuroprotection. At the same time, we have proved the possibility of using this humoral pathway to transfer tolerant phenotype to non-conditioned individuals. RIC stimulates the blood cells (BCs) via their transcriptional/translational pattern of protein synthesis, which ensures the induction of tolerant phenotype in the recipient following the transfusion of those RIC-treated cells. These findings motivated us to verify changes in the BCs paracrine activity following RIC stimulation and the possibility of using this altered cell-free secretome to improve the stroke outcome.

Tolerant secretome was prepared by incubating RIC-stimulated BCs in artificial plasma and tested in vitro (primary cortical neurons) and in vivo in a rat model of middle cerebral artery occlusion (MCAO). In addition, the protein profile of the secretome was analyzed to identify the secreted proteins and the bioactive component ensuring the induction of the tolerant phenotype. The increased neuronal survival mediated by the tolerant secretome was confirmed in vitro and in vivo in pre- and post-treatment of MCAO-subjected rats. Bioinformatic-based analysis revealed higher amounts of proteins released by the tolerant BCs; 29 proteins were recognized as secreted. More than half of them were involved in the biological processes of the response to the stimulus (GO:0050896) and the response to chemicals (GO:0042221). The protective phenotype was most likely mediated by the synergistic effect of multiple identified proteins, including those unique to the tolerant secretome (ceruloplasmin, D-3-phosphoglycerate dehydrogenase), and was promoted by the co-participation of several reaction pathways (the most probably post-translation protein modification, MAP2K, and MAPK activation, and platelet activation). Taken together, our results demonstrate that properly stimulated BCs could serve as a source for cell-free-based therapies of regenerative medicine.

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Clinical use of the phenomenon of ischemic tolerance

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Ischemia with reperfusion initiates a wide complex of changes of an inflammatory nature that can worsen local conditions, but also lead to dysfunction of a distant organ or the entire organism. Ischemia occurs in clinical practice during acute arterial occlusions (ischemic cerebrovascular accident, myocardial infarction and limb ischemia), where reperfusion subsequently occurs as part of treatment (thrombolytic treatment, angioplasty, surgical revascularization, operations with tourniquet application). Also common surgical procedures (organ transplantation, transfer of free lobes, cardio-pulmonary bypass, vascular operations, polytrauma) lead to ischemia-reperfusion damage.

The possibility of influencing the extent of ischemic damage and the consequences of reperfusion syndrome is still an unsolved challenge. Knowledge and the possibility of clinical influence of ischemia-reperfusion damage is still very limited. A practical problem is the fact that the transfer of experimental and animal results to clinical practice is still very problematic with controversial results.

In the lecture, the authors discuss the possibilities of using ischemic tolerance in the treatment of ischemia-reperfusion syndrome. Ischemic tolerance represents a robust internal defense mechanism and a state in which cells are resistant to the devastating effect of ischemia, which would itself lead to cell death. This is one of the forms of evolution. Cells exposed to metabolic stress or sublethal ischemia (preconditioning) become temporarily resistant to the action of subsequent lethal stress. The power of protection obtained by ischemic tolerance is surprisingly large.

In an experimental animal model, the protective effect of pre-conditioning and post-conditioning against ischemic-reperfusion damage is clearly confirmed, this fact is confirmed by countless experimental works. On the contrary, the number of works devoted to the clinical use of ischemic tolerance in humans is very limited. The results of these works are often contradictory, or clearly not confirming the potential protective effect of ischemic tolerance.

In the gerontic population, as a result of co-medication, activation of ischemic tolerance often does not occur, but in the case of traumatic injuries in young healthy people, the phenomenon of postconditioning can be fully utilized. In the given case, the injury can be considered as the first stage of activation of ischemic tolerance, and the therapeutically applied subsequent stress leads to full activation of ischemic tolerance.

The aim of the lecture is to provide a comprehensive overview of the use of the phenomenon of ischemic tolerance in practice, while the authors, based on the results of experimental and clinical works, state the possibility of direct application of double activated plasma from healthy donors as the most optimal use of ischemic tolerance in practice, while this is the only one that can lead to immediate treatment of ischemic -reperfusion damage.

Cell therapy for traumatic brain injury, current status and future plans – involvement of 14-3-3ζ

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Traumatic brain injury (TBI) is one of the major causes of injury-related mortality and morbidity around the world. TBI can cause cognitive and memory impairments, hearing loss or various neuropsychiatric and neurological diseases. It can be caused by sports-related injuries, automobile accidents, or blast-related injuries. Based on the severity of primary damage as well as the extent of secondary damage, TBI can be categorized into mild, moderate, and severe. Many efforts to treat TBI as a single pathophysiological condition did not lead to the successful results. Newly tested regenerative therapy approaches TBI using mesenchymal stem cells (MSCs) seem to be promising. Preclinical research demonstrated that transplanting MSCs have the potential to decrease secondary neurodegeneration and neuroinflammation, stimulate neurogenesis and angiogenesis, and enhance overall functional outcomes in experimental animals. Recently it was revealed that 14-3-3 family proteins seem to be closely associated with TBI. Specifically, the isoform 14-3-3ζ has been found involved in connection to the cell therapy of rat model of TBI, in which it fastened neuronal recovery of the animals via decreasing the inflammation and apoptotic processes and prevention of neurodegeneration.

In our project, we focused on the isolation, differentiation and "in vitro" characterization of mice MSCs intended for treatment of TBI. In our project we isolated adipose tissue from retroperitoneal region of mice. After harvesting of sufficient amount of MSCs, we differentiated the cells into neuron-like cells. These cells were compared with the control non-differentiated MSCs. After the treatment with specific compounds (melatonin, forskolin, IBMX), we have observed significant morphological neuron-like changes. Subsequently we analyzed the expression of neuronal markers such as Tau protein, Nestin, and Beta-tubulin III. We found, that Tau protein and Beta-tubulin III were expressed in the cytoplasm and Nestin expression was observed in the nucleus and cytoplasm of differentiated MSC. Our future goal is to further improve differentiation of MSC and prepare enough cells for the transplantation into mice after mild TBI and mice with a neurodegenerative phenotype. Animal models have been established at our institute and will be used to test the efficacy of the cell therapy, as well as detail roles of 14-3-3ζ in animal recovery after TBI.

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Mature neurons of the olfactory neurogenic region are connected with the striatum

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The subventricular zone (SVZ) on the walls of the lateral brain ventricles harbors the largest neurogenic niche in the adult rodent brain. Neuronal precursors of the SVZ proliferate, migrate to the olfactory bulb in a restricted pathway known as the rostral migratory stream (RMS), and differentiate into neurons. Regulatory mechanisms of postnatal neurogenesis in the SVZ/RMS are still not fully understood. Recent evidence suggests that neurogenesis in the SVZ/RMS could be regulated by neurons located directly in these regions. Till now, in the SVZ/RMS two cell populations showing morphological characteristic of mature neurons have been identified: nitric oxide (NO) producing neurons and neurons expressing secretagogin (SCGN). The role of both types of neurons in the regulation of neurogenesis has already been demonstrated. The aim of our work was to map possible projections of these populations of neurons in the SVZ and in the RMS. To test the hypothesis that NO and SCGN positive neurons of the SVZ/RMS are connected with the adjacent striatum, we injected the retrograde fluorescent tracer Fluoro-Gold (F-G) into this brain structure using a stereotaxic device. To verify the identity of nitrergic neurons and SCGN expressing neurons, double immunoflourescent labelings with anti-nNOS/anti-SCGN and anti-Fluoro-Gold antibodies were used. Microscopic analysis revealed the presence of F-G, administered into the striatum, in cells of the SVZ, as well as in different parts of the RMS. F-G-labeled cells in the SVZ/RMS were identified as nitrergic neurons or SCGN expressing neurons, indicating the existence of a neuronal circuit in which NO producing and SCGN expressing neurons are involved. Our morphological findings on the connectivity of nitrergic/SCGN expressing neurons of the SVZ/RMS with the striatal structure suggest the existence of neural circuit that may be involved in the regulation of postnatal neurogenesis within the olfactory neurogenic area.

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Are aged microglia capable of surprising? Responsiveness of microglial cells within the pineal gland during aging

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Microglia, the resident immune cells of the central nervous system, are constantly patrolling their environment, interacting with neighboring cells, and exchanging effectors and modulatory signals. Despite their surveillance role, deleterious signals prompt microglia to shift into stimulus-dependent reactive phenotypes. Once the insult is resolved, microglia may return to a basal state. In the circumventricular organs such as the pineal gland (PG), which lack a complete blood-brain barrier, microglia are continuously maintained in activated-like states due to persistent stimulation by diffusible mediators from the bloodstream and local molecules. Considering that microglia undergo detrimental changes during aging, we investigated the responsiveness of aged microglial cells within the 21-month-old (21 m) rat PG after two consecutive intraperitoneal injections (100 μg/kg body weight each) of lipopolysaccharide (LPS) from *E. coli* using fluorescent immunolabeling followed by confocal microscopy and image analysis. We observed a slight but significant increase in the number of $IBA1^+$ microglia, along with the formation of many cell clusters, after LPS treatment compared to the vehicle-injected group. Additionally, LPS appeared to enhance the expression of the cell proliferation marker PCNA in PG cells, including $IBA1^+$ phagocytes. Surprisingly, LPS treatment did not significantly impact on morphological parameters, such as cell size and shape descriptors, in these cells. Furthermore, we detected an elevated expression of the immunomodulatory cannabinoid receptor CB2 in the treated PG, including in $IBA1^+$ cells. These findings demonstrate that microglial cells within the 21 m PG are still able to orchestrate immune responses to harmful cues, such as LPS. Given the circumventricular nature of PG, circulating molecules are likely to induce acute inflammatory events within the gland parenchyma more rapidly than in other brain areas, leading to a faster resolution of inflammation.

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Selected genetic indicators of induced brain stroke ischemic tolerance found in human blood: A quantitative analysis

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Stroke stands as the second leading cause of mortality in the European Union, making it a significant medical concern. Carotid stenosis accounts for 15% of all ischemic cerebral strokes. However, there currently lacks a reliable screening method for carotid disease. Utilization of DNA analysis from peripheral blood is increasingly prevalent in diagnosing various diseases. Moreover, the potential therapeutic approach of inducing tissue tolerance to ischemia has primarily been investigated in animal models (Furman 2023). This study aims to investigate alterations in the expression of specific brain ischemia markers subsequent to carotid endarterectomy, hypothesized to activate ischemic tolerance. During carotid endarterectomy, temporary blockage of the internal carotid artery occurs. Through RT-qPCR analysis, we identified changes in peripheral blood after sub-lethal cerebral ischemia induced by carotid endarterectomy, focusing on early reported gene indicators of brain ischemia (ADM, CDKN1A, GADD45G, IL6, TM4SF1). Patients undergoing the surgical procedure were categorized into three groups: asymptomatic, symptomatic, and those undergoing carotid endarterectomy after an acute stroke. Outcomes were compared with those of a negative control group. Carotid endarterectomy impacted all monitored indicators. Significant differences (p-value 0.05-0.001) were observed when comparing the groups, indicating evidence of brain tissue's resistance to ischemic episodes. In summary, following carotid endarterectomy, symptomatic patients displayed alterations in ADM, GADD45G, and TM4SF1; acute patients exhibited changes in GADD45G and IL6, while the asymptomatic group showed modifications in CDKN1A and ADM.

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Reference:
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Studying unconstrained behavior of mice in a labyrinth to explore cognitive deficits

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Neurodegenerative disorders represent the most common causes of dementia worldwide. The underlying neural mechanisms linking distinct molecular changes and morphological changes to behavioral and cognitive deficits, however, remain unknown. Behavioral changes in early stages of neurodegenerative disorders can be subtle and commonly used behavioral tasks struggle to reveal such changes in various animal models of brain disorders. Studying unconstrained behavior of animals provides a rich environment for exploring cognitive deficits associated with various disorders of the brain. Observing mice navigate, explore, and learn freely in a complex environment allows for a more comprehensive assessment of their cognitive abilities than using more common constrained tasks which may limit behavioral expression. The resulting nuanced understanding of behavioral changes would lead to a better understanding of changing neural mechanisms between healthy and diseased brains.

To study unconstrained behavior of mice we have adapted a recently introduced labyrinth and estimated the dynamics of learning in mice searching for rewards. The labyrinth (60 cm x 60 cm) was made of acrylic semi-transparent to infrared light. Experiments were conducted in a dark room, the labyrinth was illuminated by two infrared LED arrays, animal's trajectory was tracked using an infrared camera and Bonsai software, and behavior was monitored using a Bpod system. During the initial phase of testing, wild-type mice were allowed to freely explore the labyrinth connected to a mouse cage during several consecutive sessions. After learning the position of a water reward, mice quickly learned to switch between reward ports placed at two adjacent positions separated by a labyrinth wall. Switching between ports required learning the shortest path between them. Mice appeared to adapt quickly even after rotation of the maze when the position of the water reward has changed relative to the entry to the labyrinth.

Studying complex unconstrained exploration in mice offers a window into understanding cognitive deficits in neurodegenerative disorders. We suggest that evaluating such unconstrained behavior in wild-type mice and mouse models of neurodegenerative disorders could reveal cognitive impairments even in early stages of the disorders, and might, in turn, provide insights into the underlying neural mechanisms.

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Inhibition of aldosterone synthesis during the stress-hyporesponsive period results in negative neurobiological effects in juvenile rats

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In the developing rodent, there is a period (postnatal day (PND) 3–14), called the stress-hyporesponsive period, when pups show reduced capacity to secrete corticosterone in response to several stress stimuli (1). We found that rat pups show increased rather than reduced response of the mineralocorticoid hormone aldosterone to several acute stress stimuli throughout this developmental period (2). We tested the hypothesis that the inhibition of aldosterone synthesis during the stress-hyporesponsive period results in altered behavior and modified neuroendocrine response to stressors in juvenile rats. Newborn Sprague-Dawley rat pups (males n=40, females n=41) were treated with aldosterone synthase inhibitor FAD286 (30 mg/kg per day, orally) or vehicle from PND 3 to PND 9. They were behaviorally tested in an open field (PND 23) and elevated plus-maze (PND 29) and half of them were exposed to restraint stress for 120 min (PND46). Results showed that in 10 days-old pups, treatment with FAD286 resulted in increased gene expression of CYP11B2 (aldosterone synthase) and CYP11B1 (11-beta-hydroxylase) in the adrenal glands, increased plasma levels of corticosterone, and decreased concentrations of plasma aldosterone. Postnatal FAD286 treatment increased anxiety-like behavior in female rats, not male rats. In juvenile rats treated with FAD286 postnatally, the rise in corticosterone concentrations in response to restraint stress was much more pronounced than in vehicle-treated ones, regardless of sex. Obtained results demonstrate that the lack of aldosterone in the early postnatal period as a consequence of aldosterone synthase inhibition influences anxiety-like behavior in female, but not male juvenile rats. Blockade of aldosterone synthesis during the stress-hyporesponsive period in newborn rats has consequences on stress hormone release later in life.

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How to evaluate the efficacy of thrombolysis in vivo

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Background: If a clot is visualized in an animal, then biological effect of thrombolytic drugs could be assessed directly by measuring thrombus size in thromboembolic animal models. Study validates a novel systemic embolization in vivo model using micro-fluoroscopy imaging for to evaluate the efficacy of thrombolysis in vivo.

Aims: 1/ To quantifying the thrombolytic efficacy of alteplase (rtPA); 2/ to verify the yield of multiple clots application; 3/ to describe the mortality rate and technical complication of the model.

Methods: Pilot validation study. Twenty-six outbreed Wistar rats, randomized into two groups, received three human-fibrin based clots (AC artificial clot) labelled with BaSO4 into the descending aorta. Using micro-fluoroscopy imaging for 60 minutes, the size of detected clots, after administration of rtPA (0.9 mg/kg/hour) in 16 rats (analysed 33 ACs) or saline solution (rats 10 analysed ACs 21) in an equivalent volume and time, were measured. For model validation, the relative change (%) in the clot area, lysis rates (%/min), and area under the curve (% × min) were calculated. Validated models were used in next studies with different dose of rtPA (rats 19, ACs 40), tenecteplase (rats 27, ACs 47), plasminogen addition (rats 49, ACs 99), novel mutant of rtPA - ALT04 (rats 30, 54).

Results: Clots were detected in each animal (n=132), with the mean 2.136 clots per animal. No animal died. The relative change in clot area at 60 minutes from baseline in the treated vs. control group was -46±26% vs. -21±14%. The average lysis rate in the period 0-60 minutes was -0.9±0.5 %/min (95%CI [0.7 to 1.1]) in the treated group and -0.4±0.3 %/min (95%CI [0.3 to 0.6]) in the control group. The area under the thrombolytic curve in the time 0 to 60 minutes was -662 % × min in the control group (95%CI [-866,6 to -456,5]) vs. -1433 % × min in the treated group (95%CI [-180556 to -1232,9]).

Conclusions: The model with C-arm micro-fluoroscopy imaging visualized clots in animals, allowed measurement of clot size and was sensitive to detect thrombolytic activity of Alteplase. Such model holds promise to be used for translation of new thrombolytics in preclinical research into clinical practice.

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In search for an optimal tool for detection of apoptosis in nervous tissue: Cleaved caspase-3 is present in the majority of glial cells in the intact rat spinal cord during postnatal life

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Cell death is an essential process that occurs during the development of the central nervous system. Despite the availability of a wide range of commercially produced antibodies against various apoptotic markers, data regarding apoptosis in intact spinal cord during postnatal development and adulthood are mostly missing. We investigated apoptosis in rat spinal cord at different stages of ontogenesis (postnatal days 8, 29, and 90). For this purpose, we applied immunofluorescent detection of two widely used apoptotic markers, cleaved caspase-3 (cC3) and cleaved poly (ADP-ribose) polymerase (cPARP). Surprisingly, we found significant discrepancy between the number of $cC3^+$ cells and $PARP^+$ cells, with a ratio between 500:1 and 5,000:1 in rat spinal cord at all postnatal time points. The majority of $cC3^+$ cells were glial cells and did not exhibit an apoptotic phenotype. In contrast with *in vivo* results, *in vitro* analysis of primary cell cultures derived from neonatal rat spinal cord, treated with the apoptotic inductor staurosporine, revealed a similar onset of occurrence of both cC3 and cPARP in cells subjected to apoptosis. Gene expression analysis of spinal cord revealed elevated expression of the *Birc4 (XIAP)*, *Birc2* and *Birc5 (Survivin)* genes, which are known potent inhibitors of apoptosis. Our data indicate that cC3 is not an exclusive marker of apoptosis, especially in glial cells, due its possible presence in inhibited forms and/or its participation in other non-apoptotic roles. Therefore, cPARP appears to be more appropriate marker to detect apoptosis.

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iNets - novel human neural networks to study neurodegenerative diseases

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative diseases. Pathogenesis of these TDP-43 proteinopathies cannot be studied in animal models due to human-specific TDP-43 mRNA binding targets. Therefore, we generated human iPSC-derived, colony morphology neural stem cells (iCoMoNSCs). Differentiated iCoMoNSCs formed self-organized long-lived neural networks consisting of glia and synaptically connected and electrophysiologically active neurons - iNets. Overexpression of wild-type TDP-43 in a minority of iNet neurons led to its progressive fragmentation and aggregation, resulting in neurotoxicity. scRNA-seq revealed misregulation of synaptic protein NPTX2, the mRNA levels of which are controlled by TDP-43 binding on NPTX2 3'UTR. When NPTX2 was overexpressed in iNets, it exhibited neurotoxicity, whereas correcting NPTX2 misregulation partially rescued neurons from TDP-43-ignited neurodegeneration. Importantly, we confirmed NPTX2 misaccumulation in ALS and FTLD patient neurons with TDP-43 pathology. Our work directly links TDP-43 misregulation and NPTX2 accumulation, thereby indicating a new pathway of neurotoxicity. Next, we plan to utilize iNets and MacNets (iNets integrated with iPSC-derived microglia) to study the mechanisms and consequences of TDP-43 aggregation, their spread from neuron to neuron and the link between these events and TDP-43 loss of function.

The impact of traumatic spinal cord injury on the expression of Angiotensin II receptors within the hypothalamus-pituitary-adrenal axis

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Angiotensin II as an important stress hormone can modulate the activity of the hypothalamus-pituitary-adrenal gland (HPA) axis and sympathetic nervous system through activation of its receptors. However, traumatic spinal cord injury (SCI) can disrupt the sympathetic innervation of adrenal gland and dysregulate HPA axis function which subsequently contribute to altered endocrine regulation. Since Angiotensin II receptors are expressed within organs of the HPA axis, it seems that the expression of both AT1 and AT2 receptors could be significantly impacted by the disruption of the sympathetic innervation. Therefore, the level of circulating Angiotensin II and the expression of its major receptors within the HPA axis have been studied during 28 post-traumatic days in response to severe spinal cord compression (40g weight for 15 min) induced at higher (Th1) and lower (Th9) thoracic spinal level. The observed changes were correlated with alteration of plasma level of stress hormones including corticotropin-releasing hormone, adrenocorticotropic hormone, corticosterone, epinephrine, norepinephrine and aldosterone.

During the first three days, there was detected an elevated concentration of circulating Angiotensin II that then gradually decreased to the control values. Angiotensin II receptors levels within the HPA axis as well as stress hormones fluctuated dynamically during 28 days survival period. Spinal cord trauma at higher thoracic level significantly decreased the levels of both Angiotensin II receptors.

We can conclude that the extent of sympathetic denervation of adrenal gland caused by SCI can affect neuroendocrine regulation and the expression of Angiotensin II receptors within the HPA axis.

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Body weight-supported treadmill training in spinal cord injured rats

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The goal of therapeutic interventions for spinal cord injury (SCI) is to recover both sensory and motor function, specifically aiming to restore locomotor function. In the absence of innovative treatment for SCI, rehabilitation exercise has shown promise in promoting recovery and functional improvements for individuals living with SCI. While complete SCI may not be entirely reversible, locomotor training can impact morphological, nutritional, and synaptic changes in intact motor neurons below level of injury, thereby playing a crucial role in promoting motor functional restoration.

This study was aimed to investigate the effect of body weight-supported treadmill training on the recovery of locomotor functions in rats with Th9 spinal cord injury. Rehabilitation has started 1 week after Th9 compression. The exercise schedule followed a regimen of 20 min/daily, 5 day per week, for a total training time of 11 weeks. Treadmill speed was 7cm/s. The motor activity of hind limbs was evaluated using the BBB motor score (Basso-Beattie and Bresnahan locomotor rating scale) and Catwalk test. After the 11-week rehabilitation training, the survival and regeneration of excitatory (glutamatergic) and inhibitory (GABAergic) neurons in the ventral horns of the spinal cord were analyzed using immunohistochemical methods.

The results showed that systematic and targeted training on a treadmill with body weight support can significantly contribute to the improvement of motor functions after spinal cord injury. The BBB testing showed that rats subjected to treadmill training exhibited significantly improved locomotor recovery starting at 15 days after SCI compared to non-training rats. This finding was also confirmed by the Catwalk test. Immunohistochemical analyzes revealed an increased number of GABAergic neurons in the ventral horns of the spinal cord of trained animals. These inhibitory neurons play a crucial role in the modulation of motor neuron activity that ultimately excites the corresponding muscle. No changes were observed in the number of glutamatergic neurons.

Results of this work indicate that locomotor training can be an effective therapy to promote the recovery of motor function after spinal cord injury. It represents, in combination with other pharmacological/non-pharmacological therapies, great potential in the treatment of SCI. However, further research is needed to confirm these hypotheses.

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Modulation of the gut-spinal cord axis by probiotic treatment in the Th9 compression model

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The inflammatory response that ensues after spinal cord injury (SCI) is mediated by many factors with antagonistic pro- and anti-inflammatory properties which act simultaneously on resident and infiltrating immune cells. One of the promising approaches in SCI-managing could be the targeted modulation of the gut microflora and restoration of intestinal homeostasis, which have recently been associated with neuroregeneration and neurological function improvement. The aim of this study was to regulate the inflammatory response early after SCI using the probiotic strain *Lacticaseibacillus paracasei Ž2* (5×10^9, CFU/mL), which is characterized by promising immunoregulatory properties (e.g. inhibitory activity against bacterial pathogens, biofilm formation, systemic and local intestinal immunity stimulation). Selected pro- and anti-inflammatory transcripts were analyzed in the spinal (the lesion site and adjacent rostro-caudal segments; 0.3 cm) and jejunal tissues of rats subjected to Th9 compression (40 g/15 min) and daily probiotic supplementation lasting 7 and 14 days. The results showed that markers specific for microglia/macrophages (CD11b), reactive microglia and astrocytes (CD68, IL-6, iNOS and C1q), and for TLR/NF-κB signaling pathway, playing a crucial role in injury-induced inflammation, were significantly downregulated in all monitored tissues as early as the 7^{th}-day after probiotic treatment. In the spinal cord, the effect of two-week probiotic therapy was region and target-dependent with an upward trend from the lesion site to the cranial segment. The same therapy had a more pronounced effect in the jejunum than a shorter treatment. In addition, probiotic therapy significantly stimulated the neuroprotective milieu in both tissues (gene expression ratio in favor of CD206, SOCS3 or Ptx3), supported functional remodeling and ameliorated neurogenic bowel and bladder symptoms in the first days after injury. This study shows the potential of the probiotic strain *L. paracasei Ž2* in managing SCI-induced inflammatory events in both the gut and affected spinal cord tissue during the subacute phase.

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Evaluation of the analgesic potential of recombinant GABAergic hiPSCs and their exosomes in a model of spinal cord injury induced pain

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Spinal cord injury (SCI)-induced chronic pain presents a therapeutic challenge. Recombinant cell-based therapy can provide sustained and targeted delivery of analgesic compounds, while cells can functionally integrate with the host tissue. Dysfunctional GABAergic signaling and calcium-dependent release of pain neurotransmitters are among major pathologies underlying chronic pain. Our previous studies showed that spinal transplantation of rat GABAergic progenitors releasing an FDA approved calcium channel blocker conotoxin MVIIA can attenuate injury-induced hypersensitivity in rats. Human induced pluripotent stem cells (hiPSCs) enable autologous cell derivation from readily accessible sources like blood or skin. hiPSCs also produce modulatory factors released in the form of exosomes, which have shown promising outcomes in attenuation of chronic pain. The goals of the proposed study are to explore i) the analgesic and translational potential of hiPSCs derived recombinant GABAergic cells and ii) an early intervention with hiPSCs-derived exosomes for attenuation or prevention of chronic pain development. Fibroblast and blood-derived hiPSCs were used to generate GABAergic neuronal cells. Cells were transduced by AAV2/8 MVIIA construct developed by our lab to generate recombinant cells. Both naïve and recombinant cells were grown in sufficient quantities to allow isolation of exosome fractions using ultracentrifugation. The analgesic potency of naïve and recombinant GABAergic hiPSCs and their exosomal fractions were evaluated in clip compression model of SCI pain. Sprague Dawley rats were used in the experiment. Cells were grafted intraspinally at 4 weeks post SCI and rats were evaluated weekly for mechanical and thermal hypersensitivity. In a separated group, exosomal fractions were injected intravenously at 24hours post SCI with a booster injection at 1 week post SCI. Our results showed GABAergic phenotype and the presence of MVIIA in all transduced cell lines. Exosomal fractions were isolated from selected cell lines and characterized by Nanosight. Attenuation of hypersensitivity was detected in animals grafted with GABAergic hiPSCs with stronger effect observed in the recombinant group. The effect was mitigated by anti MVIIA or bicuculine injection. An early injection of exosomal fractions followed up by a booster partially attenuated development of tactile hypersensitivity compared to a single injection. Our data suggests the beneficial effect of recombinant GABAergic hiPSCs in the management of chronic pain and possible attenuation of chronic pain development using exosomal infusion.
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The University of Miami and J.S. and S.J. hold rights to intellectual property used in the study and may financially benefit from the commercialization of the intellectual property.

Significance of animal models of mental disorders: experimental and clinical point of view

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The translation of knowledge obtained in animal models to humans is a difficult process. Nevertheless, animal models of human disease are very useful tools for investigating pathophysiological mechanisms and unravelling new treatment targets that cannot be discovered by alternative approaches (Homberg et al. 2021). However, animal models of human disease have many limitations and they rarely mimic the human disease state completely. This is particularly true for preclinical research devoted to neuropsychiatric disorders, as we cannot model the disorders themselves but only some of their signs and symptoms. The significance of the results obtained is often differentially perceived by experimental researchers and those from clinical practice. We have analysed our own experience from animal studies related to psychiatric disorders by confronting the opinions of an animal physiologist and a clinical psychiatrist. We have realised that even contradictory opinions can be explained and understood if these views are combined. We may conclude that in the fields of psychiatry and neurology, it is desirable to create research teams with both experimental and clinical researchers who are listening to the points of view of each other.

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The tryptophan-kynurenine pathway- therapeutic strategy for neuroprotection in tauopathies

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that deteriorates cognitive functions, characterized by the intracellular accumulation of abnormal filaments of the microtubule-associated protein tau, amyloid-β plagues (Aβ), and neuronal apoptosis in the central nervous system (CNS) releasing pro-inflammatory cytokines with hyper-reactive brain immune cells (microglia and astrocytes). Moreover, these deposits are associated with systemic inflammation and disruption of the many signaling pathways including the kynurenine pathway. The tryptophan-kynurenine pathway (TKP) is functionally maintained by the catabolism of the essential amino acid, L-tryptophan. This pathway generates various metabolites specifically the kynurenic acid (KA), a neuroprotective metabolite in the CNS. In neuroinflammatory conditions, under the influence of inflammatory cytokines (INF-ϒ) and reactive oxygen species (ROS), a key regulatory enzyme indoleamine 2,3-dioxygenase (IDO) is induced, declining the neuroprotective metabolite levels and increasing the production of a neurotoxic agent such as Quinolinic acid (QA). The IDO-induced QA over-stimulates the excitatory receptor N-methyl-D-aspartate (NMDA) acting as an agonist whereas, KA acts as an antagonist of this receptor modulating the functioning of the brain. We aim to screen analogs of kynurenic acid having similar biological activity, to modulate the neuroinflammation in the CNS and its functions using an in-vitro blood-brain barrier (BBB) model as well as an in-vivo model using the transgenic rats, thereby providing the new possibility that can lead to its effective use under neuroinflammatory conditions.

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The impact of subpial administration of Chondroitinase ABC on glial scar components following SCI

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Although the glial scar has a protective function in the early stages after spinal cord injury, it becomes a significant issue later on. This is because it forms a physical barrier that prevents nerve fiber overgrowth through the lesion site, hindering functional recovery and nerve tissue sensitivity. Neuronal regeneration is inhibited by the release of various inhibitory molecules, primarily chondroitin sulfate proteoglycans (CHSPGs). Our study aimed to examine the behavior of glial scar components (CHSPGs, astrocytes and microglia/macrophages) after Th9 compression (40g/15 min) and subsequent treatment with the chondroitinase ABC enzyme (ChABC) at the lesion site and in adjacent spinal cord sections in adults Wistar rats. A significant increase in the expression of CHSPGs (NG2, Neurocan and Phosphacan) was observed during the initial two weeks after compression, followed by a subsequent decline to control values. This period was identified as the optimal time for therapeutic intervention because it coincides with the maturation, stabilization, and physical barrier function of the glial scar. Two weeks after injury, we administered ChABC (0.2U in 10µl) subpially, ensuring accurate enzyme delivery to the injury's epicenter while avoiding tissue damage. Twenty-four hours after ChABC enzyme treatment, there was a significant reduction in mRNA expression of all CHSPGs compared with untreated animals in all examined segments; the lesion site and cranial segment were the most affected. One week after treatment, the effect of the ChABC enzyme no longer persisted. The reduction in CHSPG expression at the lesion site 24 hours after treatment strongly correlated with the decrease in mRNA expression of cytokine genes produced by reactive astrocytes (GFAP, S100, C3, TNFα, C1q). Microglia responding to injury were also reduced 24 hours after treatment at the lesion site (Iba, Cx3Cr1). Simultaneously, a clear correlation was observed between the reduction of CHSPGs and an increase in the expression of genes representing microglia producing pro-inflammatory cytokines (Il1b, iNOS), allong with a decrease in the expression of genes characteristic of microglia producing neuroprotective cytokines (CD206, Il1rn, TGFb, Il4Ra). The subpial application of the ChABC enzyme, administered at the appropriate time, has been shown effective in modulating the levels of CSPGs and the polarization of individual components within the glial scar post-SCI. However, a more stable modification of enzyme is needed to make the effect last longer.

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Remote ischemic conditioning modulates the inflammation after stroke in the rat model of hyperinflammation

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In 2019, stroke was the second leading cause of death and disability worldwide. In present, the FDA-approved treatment of stroke in clinic is thrombolysis using recombinant tissue plasminogen activator (rt-PA). However, efficiency of this treatment could be affected by coagulopathy and inflammation, what is an important factor for many bacterial and viral infections, COVID-19 including. Therefore, the increased demand for developing novel non-pharmacological therapies to elevate the tolerance to ischemia has been paying attention. Remote ischemic conditioning (RIPC) meets these conditions because of the ability to stimulate the endogenous protective mechanisms ensuring resistance to stroke. Our previous results confirmed efficiency of RIPC in reduction of infarct size, even in the presence of hyper-inflammatory response. In the current work, we examined the effect of RIPC on the systemic inflammation.

Hyper-inflammation was induced by lipopolysaccharide (LPS) *intratracheal* administration. We induced focal ischemia after one day of LPS intoxication (development of hyper-inflammatory reaction). RIPC was applied one hour before (pre-conditioning) and after (post-conditioning) the brain ischemia in the form of hind limb ischemia. We investigated the modulation of inflammation by RIPC during the early (3 hours) and delayed phase (24 hours) of postischemic reperfusion. We monitored the effect of this therapy on the sedimentation rate, hematocrit, clotting time and systemic oxidative stress. Moreover, we observed the changes in plasma cytokine profile.

Our results showed that RIPC affects the inflammatory response to the LPS administration and ischemia induction positively. The pre- or post- conditioning of stroke animals results in almost identical systemic response to treatments showing the early (3 hours of reperfusion) and delayed (24 hours of reperfusion) reply. We observed significantly improved sedimentation rate and hematocrit and reduction of systemic oxidative stress. RIC treatment transiently improved clotting time in early stage of postischemic reperfusion, however loss of its efficiency was observed at one day. At 3 hours of postischemic reperfusion, plasma level of hyper-acute chemokines CINC-1, LIX and RANTES increased transiently, also in conditioned animals. However the level of all of them dropped during the delayed phase, following the RIPC treatment significantly.

On the base of our results, we can conclude that RIPC should influence the systemic inflammation, what predispose this approach as additional treatment of stroke in patients with ongoing hyper-inflammation.

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Unlike in other vertebrates, majority of cerebrospinal fluid-contacting neurons in the spinal cord of C56Bl/6N mice is present in ectopic position

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Cerebrospinal fluid-contacting neurons (CSF-cNs) represent a specific group of neurons localized in close vicinity of the ependymal lining surrounding central canal of the spinal cord and brain ventricles.

However, this fact does not apply to the mouse strain C57BL/6N. Specifically, only in this strain we identified the CSF-cNs localized in an ectopic position, which is exceptional compared with several mammalian species tested. The fact is even more interesting since the genetically closely related mouse strain C57Bl/6J does not share the phenotype. On the other hand, the close genetic relationship and availability of genomic data from both strains enabled us to reveal genetic markers associated with this trait. An *in silico* comparison of the both genomes showed hundreds of variations between the both genomes. Within them, 236 variations represented homozygous sequence variants. However, only small portion - 39 (34 SNPs, 2 small indels, and 3 structural variants) targeted known protein coding DNA sequences. Comparison with another mice strain strain (Balb/C, with standard CSF-cNs localization) showed that only 20 variants were specific for C57Bl/6N. Majority of the polymorphisms resulted in missense mutations. The final 5 candidate genes studied in the segregation analysis (*Crb1*, *Cyfip2*, *Adamts12*, *Plk1* and *Herpud2*) were selected according to their expression in spinal cord, severity of mutation on protein function and conservation of the candidate gene/protein in mice strains with standard C57Bl/6N localization. Mating of two divergent inbreed lines (C57Bl/6N × Balb/C, ectopic × standard) gave rise to consistent heterozygous F1 progeny, that showed more or less wild-type CSF-cNs arrangement. Then, according to the segregation of phenotypes in the F2 progeny we found that more than one factor is required for development of proximal localization of CSF-cNs in the spinal cord. Further sequencing of segregating F2 progeny showed that the only relevant genotypes associated with increased median of distance of CSF-cNs from the wall of central canal are $Crb1^{6N/6N}Cyfip2^{6N/6N}$ and $Crb1^{6N/6N}Cyfip2^{6N/+}$.

Our study shows important role of Crb1 and Cyfip2 proteins for proper localization of CSF-cNs in spinal cord. The detected anatomical divergence in the C57BL/6N strain is also a reminder that the choice of the used model for research should be carefully considered.

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Blood cell-derived secretome as an alternative stroke treatment approach for obese individuals

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Currently, a very promising neuroprotective strategy for stroke treatment is remote ischemic postconditioning (RIPostC). This method involves inducing ischemic tolerance by applying sublethal ischemia to a distant organ or limb, leading to the activation of various endogenous neuroprotective mechanisms. Despite its positive effects, RIPostC´s clinical effectiveness is limited by factors such as age, gender, or obesity - one of today´s public health problems. Therefore, current research focuses on cell therapy, specifically on the use of cell-derived secretome, which plays a significant role in paracrine signaling. In our study, we focused on the concept of utilizing blood cell-derived secretome as a potential therapy for obese individuals. 12-week-old lean healthy Wistar rats underwent RIPostC consisting of three cycles of 5-minutes of ischemia and reperfusion. Subsequently, the blood from RIPostC-stimulated Wistar rats was processed into secretome and intravenously injected into obese ischemic rats. Our findings showed a significant reduction in infarct volume of obese rats which was positively correlated with the neurological score improvement 24 hours after reperfusion. In addition, injection of tolerant secretome successfully inhibited DNA damage in the lymphocyte cells of obese animals, while also demonstrating a significant increase in certain antioxidant enzymes. Furthermore, the typical characteristic of RIPostC mediated ischemic tolerance - the reduction in tissue glutamate excitotoxicity along with a simultaneous elevation in blood glutamate levels - was observed in those rats. We can conclude that the intravenous administration of the secretome, derived from the blood cells of healthy individuals treated by RIPostC, activates endogenous mechanisms of neuroprotection in obese animals and fully substitutes the missing effect of limb ischemia cycles of RIPostC.

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Processing of nociceptive peripheral input in spinal cord networks

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In this work we have centered on a better understanding of processing of peripheral afferent inputs in lamina I and X by dorsal horn networks, descending pathways and microglia using *ex-vivo* electrophysiology, optogenetics and 2P imaging. Lamina I spino-parabrachial neurons (SPNs) receive peripheral nociceptive input, process it and transmit to the supraspinal centres. We have found that the vast majority of the SPNs received a few direct nociceptive C-fiber inputs and generated one spike in response to saturating afferent stimulation, thus functioning as simple transducers of painful stimulus. However, majority of afferent stimulation-induced action potentials in the entire SPN population originated from a small fraction of high-output neurons. These neurons amplified and integrated the nociceptive input gradually encoding its intensity into the number of generated spikes thus far encoding an emotional aspect of pain.

Several brain regions contain neurons projecting to lamima I and X of spinal cord (SC) that is critical for the descending regulation of nociceptive pathways. Unfortunately, little is known of how these projections control the peripheral nociceptive inputs and local neuronal network in these laminae. Here, we show that activation of descending tracts controls both primary afferent-inputs and local networks in both lamina I and X.

Recent studies suggest that neuropathic pain is associated with a robust upregulation of complement effectors in the spinal cord, which ultimately leads to production of a highly active complement product, C5a. Here, we have elucidated C5a-dependent spinal mechanisms that contribute to the development of neuropathic pain induced in mice by spared nerve injury (SNI). We have demonstrated that C5a/C5aR1 signaling results in an increased intrinsic excitability of dorsal horn neurons likely via microglial activation and plays an important role in SNI-induced neuropathic pain. Overall, our findings demonstrate complex mechanisms spinal nociceptive processing in both norm and pathology.

Neurotrophic factor expression in different microenvironments six weeks after thoracic spinal cord injury and carnosine treatment

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Traumatic spinal cord injury (SCI), along with therapeutic interventions, triggers a cascade of pathophysiological processes that determine the fate of neuronal recovery. The current study investigated how 6-weeks carnosine supplementation at a dose of 50 mg/kg/day before (CBI) or after (CAI) SCI affects the cellular expressions of brain- and glial cell line-derived neurotrophic factors (BDNF, GDNF) in the neuronal and muscle microenvironments below and above the injury. Zucker- fatty rats were divided into naive (NC) and carnosine-treated control (CC), Th9 compression untreated (SCI), and carnosine applied before (CBI) or after injury (CAI) groups.

Compared to both controls, the protein concentrations of BDNF and GDNF of all injured animals, i.e., with or without treatment, were decreased in the lumbar spinal cord segments. Unlike proteins, the gene expressions of both neurotrophic factors were increased. However, while the highest increase in BDNF mRNA was detected in both carnosine-treated groups, the greatest upregulation of GDNF was found in untreated rats. Because both neurotrophic factors are known to be produced by multiple cell types, we also evaluated the expression of cell-specific genes such as microglial CX3C-motif-chemokine receptor-1 (CX3CR1), astrocyte glial fibrillary acidic protein (GFAP), oligodendrocyte Olig2, fibroblast collagen 6, and neuronal nitric oxide synthesis (nNOS) mRNAs. Correlation analyzes between neurotrophins and cell-specific genes showed strong relationships between microglial CX3CR1, BDNF and GDNF mRNA in animals from all injured groups. But, in contrast to untreated injured rats, no correlation was found between increased expressions of BDNF, GDNF, astrocyte GFAP, and fibroblast collagen-6 genes in the CAI group. BDNF protein concentration was also decreased in cervical spinal cord segments in all injured groups. But unlike the CBI and SCI groups, GDNF protein concentration did not decrease in CAI rats. In the gastrocnemius muscle, the decrease of BDNF and GDNF protein concentration was stopped in the CAI group. In untreated rats, the oligodendrocyte Olig2 mRNA was significantly decreased in lumbar and nNOS in cervical spinal cord segments compared with control and CAI rats. The current study demonstrated the effect of carnosine supplementation on all glial cells. Moreover, its neuroprotective role was also seen in the cervico-lumbar nNOS connection. In the muscle microenvironment, the main treatment effect was observed in the gastrocnemius muscles of CAI rats.

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Comparative study of imaging methods and therapy results in diseases of the spine in dogs at the Veterinary University Hospital in Kosice

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In our study, we recorded the occurrence of spine and spinal cord diseases in 3% of all dogs admitted to our clinic during the monitored period.

In the diagnosis of neurological diseases originating from the affected spine and spinal cord, x-ray is still the most widely used because it is broadly available. In spinal cord compressions, use of a contrast study - myelography has higher predictive value for lesions in the thoracolumbar segment than the lumbosacral segment. Advanced imaging is often required to confirm and refine the complete distribution of extruded disc material within the spinal canal and to identify noncompressive lesions. Over the years, we noticed a change in the number of different breeds undergoing examination, as well as a higher number of MRI performed, which is related to the increased availability and the increasing willingness of animal owners to undergo this procedure due to the understanding of its higher diagnostic value.

In our retrospective study, during 2015-2018, we performed myelography 3-4 times more often than MRI. Between 2019-2022, we used MRI 2-times more often than myelography. This led to detection of greater numbers of spinal lesions, especially intramedullary, also to a better decision-making in terms of surgical treatment. A secondary effect of higher availability of MRI is higher success rate of our patients' therapy.

Predisposition to disc herniation reflects biomechanical forces associated with the dog's body structure, genetic factors influencing disc degeneration, environmental stress. The German Shepherd, Doberman, Rottweiler, Great Dane, Basset Hound and crossbreeds are among the most commonly affected large breeds. Thoracolumbar lesions make up the majority of cases and small breeds are most often affected, especially Dachshund, French Bulldog, Yorkshire Terrier, Cocker Spaniel, Shih-tzu, Lhasa Apso, Maltese, Beagle. With the increasing availability of MRI, we have seen a higher incidence of the use of surgical therapy. The rate of recurrence of clinical signs in surgically treated dogs averaged 25%, and 59% for conservative therapy. This higher incidence is due to the large number of predisposed dog breeds mentioned above, in which recurrence after surgical therapy is usually a new herniation of another disc. When choosing a therapy, the main factors influencing the decision are type of the disease, degree of neurological dysfunction, severity of clinical symptoms and chronicity of the disease. With medical or conservative treatment, if clinical symptoms persist or worsen within a few days, it is necessary to consider surgery as soon as possible.

Aerobic exercise-driven brain resilience: insights into hippocampal alterations of obese rats

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Although the impact of lifestyle on overall health is extensively documented and well managed, obesity is considered a significant concern in contemporary society. In addition to affecting other vital organs, obesity can detrimentally alter brain function. Specifically, hippocampal changes associated with obesity may lead to chronic low-grade inflammation, disrupted neurogenesis, and impaired neuroplasticity, ultimately affecting cognitive abilities. Since aerobic endurance training (ET) leads to numerous health benefits (favourable influence on weight, glucose control, regulation of blood pressure, and overall improvement in quality of life), it is the most recommended treatment for obesity in humans. In this study, we focused on molecular mechanisms that could reveal the potential of 6-week ET to counteract obesity-induced alterations in hippocampus of a rat genetic model of obesity. Zucker-fatty and lean rats were divided into four experimental groups: lean and obese controls, as well as trained lean and obese groups. Over a 6-week period, the rats underwent an ET program on treadmill with gradually increasing speed (from 9 m/min to 18 m/min) and duration (from 15 min/day to 30 min/day). Using qPCR analysis, we assessed gene expression changes related to inflammation, activation of neurotrophin-associated signaling pathways critical for synaptogenesis and neuroplasticity and regeneration. ET of obese rats led to a reduction in the expression of the pro-inflammatory microglial marker Iba1 and modulation of neurotrophin-associated PLCγ/PKC/CAMKII signaling. Exercised animals exhibited a preference for the PLCγ/CAMKII signaling branch downstream of PLCγ, which plays a pivotal role in memory formation and synaptic plasticity. Additionally, the concurrent increase in mRNA levels of the presynaptic marker synaptophysin further supported the involvement of ET in promoting synaptogenesis and neuroplasticity. Simultaneously we observed the upregulation of mRNAs for regenerative immature/mature oligodendrocyte markers (CNP/PLP1) and neurofilaments. Finally, a passive avoidance test has shown the improvements in cognitive functions. These findings underline the potential of ET to mitigate the obesity-induced inflammatory milieu, provide neuroprotection, and promote proper hippocampus functioning.

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Neuroprotective and anti-inflammatory effects of neuroactive steroids in model of perinatal focal cerebral ischemia

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Neuroactive steroids (NAS) have been shown to impact central nervous system function through the modulation GABAergic and glutamatergic receptors. The levels of neuroprotective NAS are pronouncedly elevated in the perinatal period. As had been demonstrated previously, synthetic analogues of NAS engage in their natural metabolism and modulating GABA and glutamatergic neurotransmission without obvious adverse effects in immature rats. Likewise, synthetic analogue of NAS had neuroprotective effect in the model of perinatal focal cerebral ischemia (pFCI), anticonvulsive effect in model of tonic-clonic seizures in immature rats and did not show neurotoxicity effect. Due to the importance of other mechanism and factors in the brain insult outcome, we aimed to evaluate an effect of 3α5β-pregnanolone glutamate (synthetic analogue of NAS) on parvalbumin positive GABA-ergic interneurons (PV) activated microglia (OX-47) and astrocytes (GFAP) in the model of pFCI.

The pFCI had been induced by the infusion of the endothelin-1 (ET-1, 40pmol/1µl, 0.25 µl /min) into the right dorsal hippocampus of 12-days-old male and female rats. All procedures had been performed under 1.5-2 % isoflurane anaesthesia in the stereotaxic apparatus. One minute after the end of infusion, anaesthesia was terminated and skin was glued by the collodium. The 3α5β-pregnanolone glutamate (PregG) at the dose 1 mg/kg was administrated intraperitoneally 5 min after the end of ET-1 infusion. Animals had been returned to their dams and transcardially perfused 24h later, brains were removed, cryoprotected, sectioned (50 µm, 1 in 5 series), stained on PV, OX-47 and GFAP, and evaluated.

Our results indicate that PregG administration following pFCI has a neuroprotective effect of on PV^+ neurons in the dentate gyrus, reduce the level of microglia activation and reduce the level of reactive astrocytes.

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Mirtazapine intake during pregnancy has only moderate effect on hippocampal excitability of the offspring

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Untreated maternal depression has a negative impact on the neurodevelopment of her fetus. Use of antidepressants during pregnancy may have adverse effects on fetus, as well. Therefore we tested the consequences of maternal depression and/or prenatal antidepressant treatment on the offspring neurodevelopment in an animal model. We have used an established model of maternal depression based on three weeks long pregestational chronic unpredictable stress. As an antidepressant we choose mirtazapine applied starting with 10th gestational day via biscuit. We focused on possible changes of excitability of hippocampal neurons as hippocampus was shown to be affected by a depression. Four experimental groups were formed: no stress + vehicle, no stress + mirtazapine, stress + vehicle, and stress + mirtazapine. Hippocampal excitability was tested either in primary culture established at the first postnatal day which reflects results of prenatal development, or in acute hippocampal slices prepared on postnatal days 11 to 13. Whole cell patch clamp was used for assessment of passive and active electrophysiological properties.

In primary hippocampal culture maternal stress resulted in significantly hyperpolarized resting membrane potential and this effects was not relieved by mirtazapine treatment. Input resistance was not significantly different between groups. Passive membrane properties were not altered in hippocampal slices. Stress significantly increased a threshold for action potential firing in primary cultures but lowered it in hippocampal slices. In primary cultures but not in slices this change was compensated by mirtazapine treatment. Stress also increased the percentage of neurons in a primary culture which exhibited spontaneous firing at the postnatal days 10-13. The mean instantaneous frequency of firing was not altered in any experimental group. Altogether, we observed moderate changes in electrical activity of offspring hippocampal neurons in early postnatal period caused by pregestational maternal stress. These changes were not compensated by mirtazapine treatment. Mirtazapine itself only moderately affected offspring neuronal activity.

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Detrimental role of hyperhomocysteinemia in the ischemic/reperfusion conditions

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Hyperhomocysteinemic conditions (hHcy) as result of genetic polymorphisms or high intake of sulfur containing proteins and low vitamins B status is considered as risk factor for acute and neurodegenerative neuronal disorders.We present here results of metabolomic, histomorphological and biochemical analysis of brain tissue as well as of blood plasma developed by homocysteine (Hcy) intraperitoneal injections or by high Met diet. This conditions induce hHcy status and initiates changes in plasma and hippocampal and cortical metabolome profile, as well alterations in biochemical, histo-morphological and behavioral patterns in rats. High Hcy metabolic supply leads to the alterations in “methylation index”, changes the one C unit metabolism and tissue redox balance. This can be thought as the main damaging and noxious effect of hHcy. Our experiments also offer basic clues into the molecular mechanisms of how hHcy if combined with ischemic/reperfusion injury (IRI) could aggravate development of concurrent neuropathological processes, such higher level of neuronal loss and also presence of amyloid deposition and hyperphosphorylation of tau protein. As hHcy is frequently manifested with hypertension in humans, preventative interventions can have an important implications for likely development of ischemic stroke and neurodegeneration and deserves deeper experimental and human studies.

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The effect of Angiotensin receptor type 2 stimulation on neuroregeneration after severe spinal cord injury

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The renin-angiotensin system, primarily through its main effector hormone Angiotensin II plays an important role in regulation of blood pressure and maintaining hydro-electrolytic balance. The effects of Angiotensin II are mainly mediated by AT1, but also by AT2 receptors. It has been shown that AT2 receptors (AT2R) have an important function in neuroregeneration processes as well. In this study, the AT2R was stimulated in an experimental model of severe spinal cord compression in adult Wistar rats. Spinal cord injury is a serious medical condition that is causing profound functional impairment of limbs movement as well as disruption of vital bodily functions, such as micturition or bowel movement. Such functional damages can significantly impact an individual's quality of life and therefore it is crucial to restrict the negative consequences of such an injury. Interestingly, significantly faster motor function recovery was observed after AT2R stimulation in comparison to untreated controls. Furthermore, the selective AT2R agonist administration increased expression of markers for myelin and axonal regeneration. Histological staining supported these findings and confirmed statistically significant increase of spared spinal cord tissue, reduced microcysts and cystic cavity formation, mainly in caudal regions. These positive effects were prevented either partially or entirely by the application of the specific AT2R antagonist. Overall, these observations strongly support the hypothesis that stimulation of AT2R exhibits a considerable potential as therapeutic intervention for spinal cord injuries.

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Siponimod shows greater efficacy than methylprednisolone and Atorvastatin in reducing neuroinflammation and promoting recovery after spinal cord injury

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The study investigated the effect of three anti-inflammatory drugs (Atorvastatin, Methylprednisolone, and Siponimod) on spinal cord injury (SCI) and the subsequent inflammatory response, focusing on the activation of microglia/macrophage and astrocytes. We aimed to understand how these drugs limit the inflammatory response and influence reactive vs. neuroprotective phenotypes of microglia and astrocytes in both subacute and chronic phases after Th9 compression (40g/15 min). Gene expression was analyzed at the lesion site, as well as cranially and caudally, at 2 and 6 weeks after SCI. Atorvastatin (5mg/kg; i.p., 7d) and Siponimod (2 mg/kg, 7d, i.p.) significantly reduced the microglia/macrophage marker (CD11b) across the cranio-caudal extent, while effect of methylprednisolone (30 mg/kg, i.p., followed 6h a 24h later by10mg/kg) was limited to the lesion site and cranially. All drugs decreased Iba1 and iNOS expression in cranial segments two weeks after injury. Siponimod showed the strongest effect in promoting tissue-regenerative microglia marker (Arg1) and reducing neurotoxic microglial markers. Arg1 mRNA was found at both time points in the caudal segment, which is known to host a detrimental environment. Siponimod treatment yielded the best results, suggesting that this modulator of the sphingosine 1-phosphate receptor could shift microglia/macrophages from a neurotoxic to a neuroprotective phenotype, thus aiding axonal and neuronal regeneration. The effect of Siponimod on neuroprotective markers such as NF-H, NF-L, PLP1, and RBFox was most pronounced six weeks post-SCI. The impact on astrocytes was also remarkable. All three drugs decreased markers for reactive astrocytes (C3, TNF-α, C1q) at 2 weeks, with Siponimod demonstrating the strongest suppression of these markers in the chronic phase. Conversely, Siponimod significantly upregulated neuroprotective astrocytes, known to up-regulate many neurotrophic factors, showing a 5- to 6-fold increase in Tgm1 and Ptx3 expression. This effect was more pronounced than with Atorvastatin and Methylprednisolone. In summary, Siponimod emerged as the most effective drug in reducing inflammatory response after SCI, promoting neuroprotective microglia and astrocytes, and supporting axonal and neuronal regeneration. It showed significant potential to reduce reactive astrogliosis and enhance neuroprotection in both subacute and chronic phases after SCI, suggesting a beneficial impact on functional outcomes.

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Exploring combination therapy with epidural stimulation and atorvastatin for spinal cord injury recovery in rat models

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The spinal cord trauma represents a serious clinical problem and improving the condition of patients is among the priorities of many scientific teams around the world. Despite progress in the understanding of pathogenesis the overall mechanism of the processes taking place in the spinal cord after traumatic intervention has not been clarified yet. Our study investigates the potential synergistic effect of regenerative therapy combining electrical stimulation with application of anti-inflammatory drug atrovastatin (ATR) following spinal cord injury (SCI). We induced SCI at the T9 segment using an isoflurane anesthesia with a compression device for 15 minutes under a 40g weight. An oscillating field stimulator (OFS) subcutaneously implanted delivered a weak electric current (50 μA), changing polarity every 15 minutes for 6 weeks to enhance axonal growth from the site of injury. Experimental animals (Wistar albino female rats) were randomly divided to four groups: SCI with functional stimulator (SCI+OFS), SCI with non-functional stimulator (SCI+nOFS), and two SCI groups that received atorvastatin in addition to stimulator for 7 days after compression injury (SCI+OFS+ATR, SCI+nOFS+ATR). Behavioral testing (hot-plate test and BBB scale) proved improvements in sensory and motor functions in animals receiving combination therapy. Using Western Blot method we analyzed protein levels for astrocytes (GFAP), neurofilaments (NF-l), newly sprouting nerve fibers (GAP-43) and oligodendrocytes (CNPase). Protein analysis showed increased levels of neurofilaments, newly sprouting nerve fibers and oligodendrocytes in the groups with applied combination and single therapy. Optical density decreased in the order SCI+OFS+ATR, SCI+nOFS+ATR, SCI+OFS and SCI+nOFS. Beyond that, the level of astrocytes proteins, as an inflammation indicator decreased more in the SCI+OFS+ATR group compared to others. Histological analysis of transverse sections showed a significant reduction in both white and grey matter after the SCI. In groups with applied therapies (SCI+OFS+ATR, SCI+nOFS+ATR and SCI+OFS) we observed an increase in cumulative white and grey matter. These findings suggest a synergistic effect of atorvastatin and OFS stimulation in promoting neural recovery post-SCI, highlighting the potential of combination therapies in enhancing regenerative outcomes.

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Evidence that PECAM-1 is a component of Reissner´s fiber produced by cells of the subcommisural organ in rats, but not in mice

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Reissner´s fiber is the extracellular structure composed of glycoproteins synthesized and released by the cells forming subcommisural organ of the brain. This structure passes along the brain ventricular system and central canal of the spinal cord, ultimately breaking down in the terminal region called *ampulla caudalis*. Evidence that developmental abnormalities of subcommisural organ and Reissner´s fiber may be associated with the body axis deformities and hydrocephalus emphasized the demand to uncover its structure and function, which is only partly understood in mammalian CNS. The aim of our study was to characterize cells forming subcommisural organ and Reissner´s fiber in Wistar rats and BALB/c mice using electron microscopy, immunolabeling and gene expression analyses. In both rodent species, subcommisural organ has been identified in postero-dorsal wall of the third cerebral ventricle as a structure composed of polarized cells with nuclei located basally, close to the posterior commissure. The apical surface of these cells is sparsely covered by short cilia. Reissner´s fiber was identified as a fibrous structure present on the ciliated cells of the third brain ventricle and on the surface of ependymal cells in spinal cord. We identified that Reissner´s fiber is immunoreactive to anti-PECAM-1 antibody in rat spinal cord, which detects glycoprotein PECAM-1 typically used as a marker of blood vessels. On the other hand, in mice spinal cord PECAM-1 has been identified only in the vasculature, while Reissner´s fiber has not been PECAM-1 positive. To verify, if PECAM-1 could be an integral component of Reissner´s fiber, subcommisural organ of both rodent species has been immunolabelled. We identified that PECAM-1 is present in the apical cytoplasm of cells forming subcommisural organ in rats, but not in mice. To test, if cells forming subcommisural organ of rats express mRNA for PECAM-1, tissue samples were isolated by laser capture microdissection and analyzed using RT-PCR. Our results proved higher expression of PECAM-1 in cells forming subcommisural organ compared to other studied areas of the brain, such as ependymal lining of third brain ventricle, hippocampus and *plexus choroideus* indicating that cells of the subcommisural organ actively produce PECAM-1. Our data represent the first evidence, that PECAM-1 is present in subcommisural organ and Reissner´s fiber of rats and underline another potential difference between two rodent species often used in neurobiological research.

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Persistent olfactory dysfunction after COVID-19: the results of remote olfactory testing and data collection in Slovakia

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Smell and /or taste loss was one of the commonly occurring symptoms of SARS-CoV-2 infections. Olfactory dysfunction (OD) was transient in most patients, but persisted for a longer time in approximately 10% of them.

The growing number of patients with persistent OD after COVID-19 in Slovakia, where olfactory testing in clinical practice is not established, prompted the creation of the project "Smell and COVID-19", in which a network of otolaryngologists also participated.

The aim of the project was to test the sense of smell in post-COVID-19 patients with persistent OD with standardized smell tests, the results of which served as an objective indicator for otolaryngologists who provided them with medical care. Due to the pandemic, testing was performed remotely. For this purpose, the website www.cuch.sk was created and smell tests were sent to patients upon request. A simple six item smell test for odor identification, the Odorized Markers Test (OMT) was used in this study. From March to June 2021, 1025 patients requested smell testing. 824 patients met the inclusion criteria, i.e. they indicated OD due to COVID-19 and OD lasted 1 month or more. 72.6% of the participants were women and 27.4% were men. Average ages of the women and men were 40.6 and 36.6 years, respectively. The duration of OD at the time of testing ranged from 1 month to 1 year. Based on the data provided by the participants, OD occurred most frequently between November 2020 and February 2021 which corresponds with occurrence of the Alpha variant (B.1.1.7) of SARS-CoV-2 related to higher risk of OD. Using OMT, hyposmia or anosmia was confirmed in 82.6% of participants. The remaining 17.4%, of participants, although determined to be normosmics according to OMT, complained of parosmia and/or phantosmia. Self-assessment of smell did not correlate with OMT outcomes of participants in all cases. That points to the relevance of psychophysical smell testing. Our study also revealed same correlations between persistent OD and the monitored parameters.

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Ultrastructural insight on the arrangement of ependymal cells, vasculature and fractone bulbs in the central canal lining of rat spinal cord

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Ventricular system of CNS is covered by ependymal cells, which are generated during the late embryonic period and early postnatal life. Even though, the ependymal cells of spinal cord have been intensively studied during the past decades in adult rodents, their postnatal development and function remain poorly understood. Moreover, the data on arrangement of ependymal cells, vasculature and fractone bulbs in spinal cord during the ontogenesis are missing. In this work, we focused on the study of ultrastructure of ependymal cells in selected time points during the postnatal life (8, 29 or 90 postnatal days). We identified that vimentin positive ependymal cells of central canal lining in neonatal, as well as adult rats, form basal processes directed toward the nearby pan-laminin positive blood vessels or *pia mater* of the ventral median fissure. Ultrastructural analyses revealed that single or even two basal processes of ependymal cells contact vascular basal lamina or its narrow extensions termed vascular fractones. At the age of P29, pan-laminin positive globules emerge at the interface between the ependymal cells and the surrounding neuropil. These structures corresponded to the labyrinths of extracellular matrix termed fractone bulbs, which were identified in this region using electron microscopy and immunogold labelling. 3D reconstructions of images obtained by serial block face-scanning electron microscopy revealed that fractone bulbs surround the basal processes of ependymal cells. We observed small vesicles in the cytoplasm of ependymal cells, as well as the globules separating from the fractone bulbs, indicating potential molecular transport between these two compartments. Since fractone bulbs are composed by laminin, we hypothesized whether laminin is produced by the ependymal cells and subsequently transported to the fractone bulbs. Screening of laminin subunits transcripts proved that ependymal cells in adult rat spinal cord express mRNA encoding laminin, mainly the α3, α5, β2 and γ1 subunits. Taken together, our data indicate that ependymal cells contact nearby vasculature in each of the studied time points and contribute to the development of fractone bulbs, which role in the spinal cord remains unknown.

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Keywords: ependymal cells, vasculature, fractone bulbs, rats

Brain myelin as an energy source

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Recent discoveries demonstrate that CNS myelin acts as an intrinsic energy store and source that supports brain metabolism in extreme conditions. It appears that neural function is preserved in the absence of glucose transport in oligodendrocytes, and that myelin content diminishes extensively and selectively in the brain of marathon runners at the completion of the effort. This myelin reduction is extensive and involves white and grey matter including primary motor and sensory cortical areas and pathways. Post-exercise rest allows myelin levels to recover fully within weeks. These findings reveal that myelin consumption and replenishment is a novel form of metabolic plasticity aimed at maintaining brain function during hypoglycaemia. Mechanistic details of how myelin lipids contribute to brain energy demand remains unknown and difficult to grasp. Obtaining this knowledge, however, may help understanding brain function decline during ageing and neurodegenerative diseases, as waning glial support leading to synaptic, axonal and neuronal malfunctions may cause fuel shortage thus leading to metabolic deficits and nervous tissue damage.

These emerging findings challenge the commonly held view that neuronal function relies exclusively on glucose and oxygen supply, as the brain has no obvious energy stores, except for limited astrocyte glycogen.

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3D Printed Scaffolds for Repair of Injured Spinal Cord and Peripheral Nerves

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Injuries of peripheral nerve (PNI) and spinal cord (SCI) are a significant medical and social problem because it is characterized by long-term limb dysfunction and a high level of disability. Treating PNIs represents a major challenge in reconstructive surgery and regenerative medicine. 3D printed scaffolds represent a promising approach for treatment of these injuries. Here we suggest a biotechnological approach for 3D printing of scaffolds with a biomimetic architecture at a spatial resolution up to a micrometer, which were used for implantation in treatment of PNI and SCI in Wistar rats. The approach is based on 2-photon photopolymerization of organic polymers and is scalable to lesion geometries. The scaffolds were implemented as multiple densely packed parallel micro-tunnels (~50 μm) running through their whole length. Having thin walls between tunnels, the scaffolds are almost hollow and simultaneously creates a large internal surface area, providing thereby a natural spatially oriented substrate for the axonal and vascular ingrowth. We have found that the scaffolds, implanted in the excision of the lateral half-fragment of the spinal cord at the level of T_{12}–T_{13}, were well integrated into the spinal cord without the formation of a significant gliofibrous scar. Axons surrounded by oligodendrocytes, as well as vessels were observed in almost each tunnel. Implantation also significantly improved the motor and sensory functions of the paretic ipsilateral limb by 4th week, and the achieved recovery was maintained until the end of 5th month after the operation. The implantation of the scaffolds in a place of dissected sciatic nerve also resulted in the functional recovery outperforming one for both hollow silicon tube and neurorrhaphy. Thus, 3D oriented hollow scaffolds having a large internal surface area creates conditions for enhancing peripheral nerve and spinal cord regeneration and recovery of the motor and sensory function of the paretic limb.

Transgenic minipig models of the serious eye diseases

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The retina is the innermost, light-sensitive layer of tissue of the eye, it develops from the embryonic diencephalon and is considered brain tissue. Neurosensory cell types split into ciliated sensory cells, photoreceptors and retinal ganglion cells. Our experimental effort is focused to gene mutations, till now incurable eye diseases. Usher Syndrome (USH) is rare genetic disorder caused by a mutation in one of 11 genes resulting in hearing loss and visual impairment. Mutation in the gene USH1c, coding protein harmonin, is responsible for the congenital deafness. Piglets bearing homozygous mutation in USH1c have the serious statokinetic phenotype, too. Both, stereocilia of the inner and outer hearing cells in organ of Corti as well as stereocilia of sensory hair cells in semicircular canals are heavily disorganized. The first symptom after birth is the circular movement of piglets that requires a special care to learn udder feeding. Moreover, the homozygote piglets do not listen the mother's signal for feeding. The retina phenotype develops slowly and it is first recognized by electroretinography and later by behavioral tests, too. The codiong sequence for harmonin (4.7 kb) is suitable for composition of AAV vectors that are used for gene therapy of vision via surgical deposition in the subretinal space. The limited spreading of vectors among photoreceptors represents the main disadvantage of this approach. The recently developed vectors penetrating the inner limiting membrane are promising solution of this obstacles. The vector introduction directly in lateral semicircular canal can improve vestibular disfunction and partly remove deafness. The USH1b mutation, causing absence of myosin-VIIA 7a (MYO7A), also affects hearing and vision. However, 6,645-bp MYO7A cDNA is too large to be delivered using standard AAV technology. The dual AAV vector systems, in which the transferred gene is split into two separate vectors that recombine in the target cell, can allow the use of AAV vectors for larger genes. Recently, the third generation of lentiviral (LV) vectors was developed. LV vectors have a high coding capacity beyond 10 kb allowing the delivery of the entire coding sequence of large genes in a single vector. The large animal model is key precondition for development of safe and efficient gene therapy. Center PIGMOD disposes with transgenic minipigs for USHER Syndrome 1C and 1B and Stargardt disease and together with a broad international cooperation it strives for the development of preclinical models for vestibulo-cochlear and vision impairments.

The effect of Siponimod on neuronal and glial cell protein markers in the rat spinal cord injury model

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The activation of resident glial cells, i.e. astrocytes and microglia in response to spinal cord injury (SCI) is a crucial mechanism accompanied by the secondary, sub-acute phase of trauma, which can harm surrounding neurones, when is overactivated. Siponimod (SIPO), currently used in Multiple Sclerosis may be a promising candidate for SCI management. SIPO prevents lymphocyte infiltration to the central nervous system, as well as it can bind to sphingosine-1-phosphate (S1P) receptors S1PR1 and S1PR5 expressed by neuronal and glial cells.

This study aimed to examine the protein level of glial cell markers, as well as alpha-synuclein (α-syn), and other proteins crucial in the proper function of neurones in the rat's spinal cord (SC) after SCI and SIPO administration.

Female Wistar Rats were divided into the three experimental groups as follows: The control group subjected to laminectomy only, the SCI group passed the Th9 segment compression with a weight of 40 g/15 min and rats after the SCI with post-surgical SIPO administration (1mg/kg i.p.). SIPO was administered 30 minutes after the compression and for six consecutive days until decapitation. Then the cranial segment (above the site of injury) of SC was isolated for Western blot analysis.

The level of IBA1 and GFAP, being strongly and specifically expressed by microglia and astrocytes, respectively, was increased after SCI. This up-regulation was abolished by the SIPO administration. Similarly, we observed a raised level of α-syn, which was partially eliminated under SIPO treatment. α-syn is a presynaptic protein crucial in neurotransmission, but when overexpressed it can gain pathologic conformation and function, which can share with the intact cells in a prion-like spreading mechanism. Simultaneously, we observed that trauma can increase Akt kinase phosphorylation on Ser473, being crucial to reach its pro-survival activity.

Current results suggest that six days after trauma in the SC both harmful processes, like an uncontrolled inflammatory response, and α-syn overexpression, as well as some compensatory mechanisms, may occur. Furthermore, SIPO has a suppressive effect on the major changes observed after trauma, which makes it a potentially promising neuroprotectant in SCI therapy.

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Genes expression altered in activation of ischemic tolerance through carotid endarterectomy in human blood

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Ischemic stroke poses a significant health threat and ranks as the second leading cause of death in the European Union. Among all cases of ischemic strokes, carotid stenosis contributes to approximately 15%. Although therapeutic approaches aiming to induce tissue tolerance to ischemia have primarily been investigated in animal models [Furman 2023], their potential benefits in humans remain underexplored. This study aims to explore alterations in the expression of genes associated with cerebral ischemia in the peripheral blood of human patients affected by cerebral ischemia and subsequently treated with carotid endarterectomy (CEA), a procedure believed to activate ischemic tolerance mechanisms. Patients undergoing CEA were categorized into Symptomatic and Asymptomatic groups based on the presence or absence of symptomatic atherosclerosis and stroke before the CEA procedure. Additionally, a third group, termed the Oxymetric group, exhibited a decrease in oximetry levels below 20% during CEA. Another group consisted of patients with leg arterial thrombosis, while the final group served as a negative control, comprising healthy individuals without a history of stroke, thrombosis, or CEA. Peripheral whole blood samples were collected from all study groups, and whole transcriptome analysis was conducted on their cellular fractions. The analysis of the Symptomatic group revealed 791 genes exhibiting a significant fold change in expression ($> \pm 2$) compared to the negative control, with 449 genes specific to this group. In the Asymptomatic group, 688 genes were identified, including 360 specific to this group. The Oxymetric group showed 637 genes, with 302 being specific to this category. In the Thrombosis group, 879 genes were detected, with 506 being unique to this group. When compared to the negative control, several genes, among the more than 20,000 tested, emerged as specific to our test cohorts. Upon overlapping between the groups, only a few dozen genes with potential involvement in the pathways associated with the induction of ischemic tolerance were identified.

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Reference:
Furman, M. et al. (2023) ‘Quantitative analysis of selected genetic markers of induced brain stroke ischemic tolerance detected in human blood’, Brain Research, 1821, p. 148590. doi:10.1016/j.brainres.2023.148590.

Proteomic analysis of signaling pathways in the rat hippocampus after delayed remote ischemic postconditioning

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Delayed remote ischemic postconditioning (dRIPostC) is a protective procedure for brain damage caused by ischemia/reperfusion (IR), but the mechanism of this treatment is not yet clear. In this study, the neuroprotective effect of dRIPostC was investigated in a rat model of cerebral IR performed by four-vessel occlusion (4VO), and the potential mechanism was assessed by proteomic analysis. Rats were divided into groups (n=5): Sham (surgery without ischemia), IR (10 min of 4VO and 3 days of reperfusion) and dRIPostC (10 minutes of 4VO and 3 days of reperfusion, interrupted by 20 minutes of ischemia caused by reversible hindlimb strangulation after 48 hours of reperfusion). The brain tissue was extracted and processed for proteomic analyses. The LC-MS/MS data obtained were analysed using Scaffold software and bioinformatic analysis was performed for three comparisons: IR vs. dRIPostC, IR vs. Sham, and Sham vs. dRIPostC. Pathway analysis results were provided using the Reactome Analysis Service. Pathways that were differentially expressed at an adjusted p-value ≤ 0.05 were considered significantly regulated. We detected 36 down and 6 up-regulated ($p \leq 0.05$) pathways in IR vs. dRIPostC; 46 down and 1 up-regulated ($p \leq 0.05$) pathway in Sham vs. dRIPostC; and 23 down and 2 up-regulated ($p \leq 0.05$) pathways in IR vs. Sham. We were mainly interested in signaling pathways with a possible neuroprotective effect. In the IR vs. dRIPostC comparison group, we detected 8 top down-regulated signal transduction pathways (e.g. signaling by Rho GTPases, RhoA GTPase cycle, RhoA activation) and 2 top down-regulated programmed cell death pathways. When comparing Sham vs. dRIPostC, we detected pathways that correlated with IR vs. dRIPostC (4 top down-regulated signal transduction pathways). The IR vs. Sham comparison revealed the top down-regulated pathways: e. g. Rho GTPase effectors, p75NTR regulate axonogenesis and RhoA activation. Our analysis showed that dRIPostC can down-regulate pathways involved in programmed cell death and thus may play a beneficial role in cell survival after ischemia. In addition, delayed postconditioning acted as a down-regulator of signaling through Rho signaling pathways. The Rho/ROCK pathway mediated by the Rho GTPase is closely associated with neuronal apoptosis and axonal growth inhibition. Inhibition of the Rho/ROCK pathway can play a key role in the stroke pathogenesis and its targeting may be critical for improving the treatment of ischemic brain injury.

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Genetic forms of Parkinson's Disease in Central Europe

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Introduction: Parkinson's disease (PD) is a neurodegenerative disorder with complex genetic background, where 10% of cases show a clear Mendelian inheritance. However, most of the genetic studies were performed in the PD cohorts of North-West parts of Europe, and there are only a few genetic data coming from Central European region. To address this gap, this study aimed to screen for the monogenic form of PD in Central Europe.

Methods: Multicentric cohort of 1612 PD patients from 9 tertiary centres in Central Europe, was screened using the microarray (n=1612) or whole-exome sequencing in early-onset and familial cases (n=220). Variants in validated and candidate disease genes and risk factors for PD and atypical parkinsonism were further analysed. All findings were confirmed by Sanger sequencing.

Results: In our cohort, we identified 27 PD carriers of the *GBA1* risk variant, 5 of the *LRRK2* variant, 3 of bi-allelic *POLG and* 1 of *PRKN* pathogenic variant, 1 was positive for the *ATP1A3* pathogenic variant. Within the exploratory analysis, we identified additional carriers of the *ATP1A3, CSF1R, DCTN1, GBA1, LRRK2, MAPT, PDGFB* and *PLA2G6* rare, potentially pathogenic variants of unknown significance. Additionally, we detected 4 PD cases of p.L1795F *LRRK2* variant, which was recently proposed as a possible strong risk factor. All 4 cases were characterised by akinetic-rigid PD phenotype with early onset of severe motor fluctuations, 2 receiving LCIG therapy and 2 implanted with STN DBS; all 4 cases showed unsatisfactory effect of advanced therapies on motor fluctuations.

Conclusion: Our data suggest that *GBA1* and *LRRK2* variants represent the majority of the PD monogenic cases in Central Europe, where the novel p.L1795F *LRRK2* may represent the most common currently known pathogenic *LRRK2* variant. Together with the ongoing clinical trials for LRRK2 inhibitors, this finding emphasises the urgent need for more ethnic diversity in PD genetic research.

The analysis of apoptotic and non-apoptotic roles of caspase-3 in cells of the rat spinal cord in the postnatal period

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Apoptosis is a process with an important role in the development, function and pathology of nervous system. During apoptotic events, a specific group of proteases known as caspases, plays a key role, however, there is an evidence that these enzymes may be involved also in non-apoptotic processes. Our previous experiments proved presence of large population of cells with cleaved caspase-3 (cC3) in the nervous tissue of postnatal rat spinal cord. We observed a high number of $cC3^{+}$ cells and a very low number of cells positive for cleaved poly(ADP-ribose) polymerase (cPARP) representing an another marker of apoptosis. Since these data raised questions regarding the role of cC3 in this region, we hypothesized that activity of caspase 3 could be strictly regulated in spinal cord. One of the potential mechanism involved in regulation of cleaved caspase-3 activity could represent inhibitors of apoptosis, known as IAPs. Therefore, we studied expression of *Birc* genes (*Birc1-Birc7*) encoding IAPs in rat spinal cord by digital PCR. The expression was studied at three stages of postnatal ontogenesis, at neonatal – P8, adolescent – P29, adult – P90 rats. Our data revealed that the most abundant gene transcripts represented *Birc4, Birc2* and *Birc5,* whose comprised more than 90 % of the entire IAP gene family. The most expressed gene was *Birc4 (XIAP)*, which contributed by more than 50 % to whole IAP gene transcripts pool at P8 and gradually rised its expression throughout the ontogenetic stages. The level of *Birc2* gene expression was stable throughout the ontogenesis, without significant changes between the studied time points. On the other hand *Birc5* transcription showed an evident decline from P8 until P90. According to our data, the activity of caspase-3 could be regulated by proteins from the IAPs family, especially by the protein XIAP encoded by *Birc4* gene or Survivin (encoded by *Birc5*), which is associated with proliferation during early ontogenetic stages. The steady presence of *Birc2* expression might be explained by the involvement of its product in multiple signalling pathways besides apoptosis. Altogether, our findings suggest possible mechanism of caspase-3 regulation in the nervous system and the need for further research of the complex regulation of apoptosis and elucidate also other non-apoptotic mechanisms of function of these proteases.

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Is Angiotensin II receptor type 2 involved in potentiated intrinsic regenerative ability in injured spinal cord?

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Axonal regeneration in the CNS is a complex process influenced by both intrinsic and extrinsic factors. The inhibitory molecules are not only critical determinant of axonal regeneration failure, but diminished intrinsic regenerative ability of mature CNS neurons also plays crucial role. Angiotensin II receptor type 2 (AT2) is abundant during the prenatal period, but its expression gradually decreases with postnatal age. Interestingly, it becomes upregulated after pathological conditions, including CNS injuries. Therefore, we studied the time-dependent receptor expression after severe spinal cord injury in order to accurate timing of pharmacological intervention.

Female adult Wistar rat weighing 250-320g were used to induce severe spinal cord compression (40g weight for 15 min) at Th9 spinal level. Injured spinal cord was studied within four weeks survival period (on 1, 3, 7, 14, 21 and 28 day). The AT2 receptors have been continually stimulated using selective high affinity ligand CGP42112, or blocked with PD123319 via osmotic minipumps. Hind limb motor function, body weight, bladder function, transcranial motor evoked potentials, spinal cord histopathology as well as the gene and protein expression of the AT2 receptor, GAP-43, neurofilaments, myelin basic protein, CNPase, myelin proteolipid protein, and Olig2 have been analysed.

Delayed and transient increase of the AT2 receptor expression was observed from 14th to 21st post-injury day. However, it normalized almost to control values 7 days later. The time period of upregulated receptor expression was selected as most accurate interval for its pharmacological activation. The improvement of monitored functional parameters, increased spinal cord tissue preservation, decreased number of cysts and cavities, and upregulation of axonal outgrowth and major structural components were detected. The AT2 receptor antagonist prevented observed beneficial effects.

We can conclude that the stimulation of delayed and transiently expressed AT2 receptors improved the observed functional parameters and potentiated the diminished intrinsic regenerative ability of severe injured spinal cord.

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Behavioral and functional consequences of maternal separation of various duration in rats

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The neurogenesis hypothesis of depression assumes that postnatal production of new neurons in the olfactory system may be causally related to depressive behaviors. To test this hypothesis, it is necessary to investigate neurogenesis in an animal model of depression. Maternal separation (MS), the postnatal disruption of mother-infant interaction, is a traumatic event that can have long-term effects on brain development and subsequent function and behavior. The aim of this study was to investigate behavioral consequences of MS of different duration in order to find an appropriate model of separation to induce depressive-like behavior. Our next goal was to find out the suitability of MS for investigation the functionality of neurons arising in the postnatal neurogenesis in the olfactory system. In the first two groups, rat pups were separated from the mother daily for 3h from the first postnatal day (P1) till P7 or P21. The animals from these groups were allowed to survive until adulthood, when they were tested in the emergence test and elevated plus maze test. The third group was designed to investigate MS-induced activation of postnatally born neurons in the olfactory bulb (OB). In this group, pups were exposed to single maternal separation (SMS) for 2h at the P21. The activation of neurons after SMS was measured by c-Fos expression. The results of behavioral tests showed that one week as well as three weeks of MS induced long-lasting significant changes in animals behavior. These changes indicate an increase in anxiety-like behavior, or can be a manifestation of increased risk assessment. Although changes in anxiety-like behavior were comparable at both durations of MS, locomotor activity was significantly reduced only after three weeks of MS. This suggests a more severe behavioral impact of MS of longer duration. Regarding functional consequence of MS, quantification of immunohistochemically labeled c-Fos+ cells revealed that exposure to SMS induced marked increase of the number of Fos+ cells in the OB, the structure dedicated to odor information processing and where expression of c-Fos protein is usually evoked by odor stimulation . Although MS is not a typical model of odor stimulation, our results indicate an involvement of olfactory system in mediation of the MS-evoked effects. In conclusion, our results suggest the suitability of three-week MS to induce depression-like behavior, as well as the relevance of two-hour SMS to investigate the functionality of newly generated OB neurons.

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Multiple roles for Angiogenin as a new clinical target for stroke

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Stroke is a medical condition that abruptly reduces blood flow to the brain resulting in brain injury affecting more than 15 million people each year worldwide, and leading to a significant number of stroke survivors with neurological deficits. With this scenario, the only available and approved treatments for ischemic stroke are the pharmacological reperfusion therapies to dissolve the clot/thrombus with tissue plasminogen activator or the mechanical approaches to evacuate the occluding thrombus (thrombectomies). Although these are effective and saving-live therapies, in general, they can only be applied to ischemic patients within the first hours of the symptoms onset. In this scenario, for a large number of stroke patients the only therapeutic options are the long-term rehabilitation programs. Therefore, it is urgent to investigate for complementary strategies supporting the current reperfusion and rehabilitation therapies. After ischemic stroke, not all cells die immediately after the blood supply interruption and there might be hypoperfused brain tissue at risk where the cells can be rescued with appropriate reperfusion or neuroprotection strategies. In this context, our group has studied the role of Angiogenin (ANG) as a potential biomarker for stroke recovery and as new acute neuroprotective therapy. As a unique ribonuclease, ANG is a small protein that shares 33% of its sequence with the bovine pancreatic ribonuclease A. Therefore, ANG (also named RNase 5) has been classified as a member of the ribonuclease A superfamily for its ribonuclease activity (the activity to catalyze the degradation of RNA into smaller components contributing to the metabolism of nucleic acids), crucial for cell bio-functions. This unique property determines its center roles in angiogenesis, cell proliferation and cell survival. The neuroprotection effects of ANG have been mainly described in neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis and Parkinson's disease, however its role in stroke disease is nowadays unclear. It will be shown how in different studies ANG increases in ischemic tissue both in mice and human brain, and in plasma after rehabilitation therapy, the second one related to better outcomes. Also, apart from pro-angiogenic actions of human recombinant ANG on endothelial cells in vitro, ANG treatment increases SVZ-derived Neural Stem Cells yields in culture and is found up-regulated after physical exercise in the ipsilateral neurogenic SVZ niche after ischemia in mice compared to control non-ischemic brains. Finally, acute post-stroke therapy in C57Bl/6 male mice undergoing transient middle cerebral artery was occluded involving the inhibition of the apoptosis signaling pathway. Overall, the presented data supports ANG as a potential treatment for acute ischemic stroke in a clinically-relevant scenario and associates its increased levels during post-stroke recovery with better outcomes.

Phenotypical, genotypical and pathological characterization of the moonwalker mouse, a model of ataxia

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Spinocerebellar ataxias (SCAs) are a group of debilitating genetic diseases characterized by cerebellar dysfunction, specifically by loss of balance and coordination. No cure is currently available for SCAs. A critical factor in the lack of available treatment is that the cellular mechanisms of SCAs are poorly understood. Two SCAs, SCA14 and SCA41, are caused by transient receptor potential channel 3 (TRPC3) gain of function. Notably, a TRPC3 gain of function mutation had been identified in a mouse ENU ataxia mutagenesis screen and dubbed the Moonwalker (MWK) mouse. In this study we undertook a detailed characterization of the natural course of the disease, the cellular pathology, and the possible disease mechanisms. Our analysis of the MWK phenotype shows a non-progressive ataxia that is first detectable around P17 and characterized by a broad spectrum of signs. The MWK mouse shows numerous behavioral symptoms including tremor, altered gait, circling behavior, impaired motor coordination, and impaired motor learning. Cerebellar pathology is characterized by early postnatal loss of unipolar brush cells (UBCs) and a slowly progressive, moderate Purkinje cell (PCs) loss after 3 months of age. Interestingly, no obvious correlation was observed between PC loss and severity of motor symptoms. This is probably due to the fact that PC function is severely impaired much earlier than the appearance of PC loss. Indeed, PC firing is already impaired in 3 weeks old mice. Notably, structural damage to PCs also precedes the PCs death by several weeks. At postnatal day 40 the synaptic contacts between PC spines and parallel fibers are severely decreased and the PC axons show signs of degeneration. Genetic analysis of 21-day old MWK cerebella shows, among other alterations, changes in neuronal development and in synaptic signaling. Our results indicate that the gain of function $TRPC3^{mwk}$ mutation affects the development and network integration of PCs and UBCs and causes PC dysfunction and ultimately PC loss. Finally, our data suggest that PCs activate specific mechanisms to defend themselves from cell death (possibly in a lobule-dependent fashion). Understanding these mechanisms might help developing innovative strategies to treat neurodegenerative diseases.

Emission of 50-kHz ultrasonic vocalizations in hemiparkinsonian rats as a new preclinical approach to study the affective properties of drugs used in the dopamine replacement therapy of Parkinson's disease

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Dopamine replacement therapy used in Parkinson's disease (PD) may induce alterations in the emotional state, which have been implicated in the manifestation of iatrogenic psychiatric-like disturbances. Preclinical investigations in this field are currently limited, since few reliable paradigms are available. To provide a relevant experimental tool in this respect, we evaluated: i) the effects of dopaminomimetic drugs on the emission of 50-kHz ultrasonic vocalizations (USVs), a behavioral marker of positive affect, in rats bearing a unilateral lesion with 6-hydroxydopamine in the medial forebrain bundle; ii) the associated modifications in neuronal activation in subcortical and cortical regions of the dopamine-denervated hemisphere. A first cohort of hemiparkinsonian rats received apomorphine (2 or 4 mg/kg, i.p.), L-3,4-dihydroxyphenilalanine (L-DOPA, 6 or 12 mg/kg, i.p.), or pramipexole (2 or 4 mg/kg, i.p.) in a test cage (× 5 administrations) on alternate days. Seven days after treatment discontinuation, rats were re-exposed to the test cage to measure conditioned calling behavior and thereafter received a drug challenge. Hemiparkinsonian rats treated with either apomorphine or L-DOPA, but not pramipexole, markedly vocalized during repeated treatment and after challenge, and showed conditioned calling behavior. Moreover, apomorphine, L-DOPA and pramipexole elicited different patterns of 50-kHz USV emissions and rotational behavior, indicating that calling behavior in hemiparkinsonian rats treated with dopaminomimetic drugs is not a byproduct of motor activation. A second cohort of rats received an acute or repeated (× 5, on alternate days) administration of apomorphine (2 mg/kg, i.p.) or L-3,4-dihydroxyphenilalanine (L-DOPA, 12 mg/kg, i.p.) and were evaluated for immunoreactivity for Zif-268, a marker of neuronal activation, in the nucleus accumbens (NAc), caudate-putamen (CPu) and medial prefrontal cortex (mPFC), which are brain regions that regulate the affective properties of drugs. Acute and repeated treatment with either apomorphine or L-DOPA stimulated the emission of 50-kHz USVs in hemiparkinsonian rats, and this effect was paired with increased Zif-268 immunoreactivity in the NAc and striatum, but not mPFC. These findings indicate that subcortical and cortical areas may differently regulate the emission of 50-kHz USVs in hemiparkinsonian rats treated with dopaminergic drugs used in the DRT of PD. Taken together, our behavioral and neurochemical results suggest that measuring 50-kHz USV emissions may be a relevant experimental tool for studying how dopaminomimetic drugs modify the affective state in parkinsonian rats.

Carotid endarterectomy – potential induction of ischemic tolerance in clinical praxis

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Stroke is the second most frequent cause of death and the most common reason for older population disability in developed countries. Stenosis of the internal carotid artery (ICA) can also contribute to stroke. Carotid endarterectomy (CEA) represents the prevention of stroke in patients with ICA stenosis. CEA is indicated in patients with asymptomatic stenosis of ICA over 60% with a presence of minimally one risk factor of stroke. In patients with neurological symptoms, CEA is indicated when ICA stenosis is over 50%. Major postoperative complications after CEA are stroke and death. The rate of major, severe postoperative complications should not exceed 3% in asymptomatic patients and 6% in symptomatic patients. CEA can be performed under general anesthesia or in locoregional anesthesia. It is essential to ensure blood flow in the ICA during clamping of carotid arteries. For that purpose, shunts are inserted into carotid arteries. More methods are used to monitor blood perfusion during the clamping of carotid arteries, such as monitoring consciousness during local anesthetics and transcranial Doppler stump pressure. Our department uses transcranial cerebral oximetry and hasgoodexperience with it. Carotid endarterectomy can be performed as a conventional or eversion.We prefer eversion endarterectomy.

We found eversion carotid endarterectomy in general anesthesia as the optimal method, and transcranial cerebral oximetry was used to save monitoring of brain perfusion during the operation.

Advancing spinal cord injury repair through multidisciplinary herapeutic approaches

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Spinal cord injury (SCI) is a devastating neurological disorder that strongly impacts the physiological, psychological, and social behaviour of those affected. The spinal cord trauma, known as "primary injury", triggers a cascade of events, termed "secondary injury", leading to further neurological damage and contributing for regeneration failure after SCI. These include glutamate excitotoxicity, a potent inflammatory response, the release of molecules that inhibit axonal growth and the formation of a glial scar. It is urgent to develop new and effective therapeutic strategies. Several important contributions for the field have been made by Silva's Lab, namely by: 1) Introducing new molecular therapies that are able to protect the neural tissue after a SCI, leading to functional recovery of paralyzed animal models; 2) Identifying new mechanistic basis of the dysfunctional regulation of the inflammatory response mounted after SCI. We demonstrate that temporal changes in the sympathetic signaling to the spleen are associated with neutrophil infiltration into the spinal cord; 3) Pioneering the use of macrophage-derived secretome as a molecular therapy of Spinal Cord Injury repair. We demonstrated that polarized macrophages-derived soluble factors and extracellular vesicles are a promising therapy for SCI; and 4) Development of novel therapies for SCI repair based on Biomaterials and Tissue Engineering. This lecture will explore recent breakthroughs from our lab, shedding light on future directions in SCI treatment and repair.

The stimulation of AT_2 receptors can promote an angiogenic response after severe spinal cord trauma

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Traumatic spinal cord injury is a serious medical condition that can result in permanent functional deficits for patients. The injury causes local disruption of vascular integrity, leading to ischemia, inflammation, and tissue damage. Therefore, blood supply recovery is crucial for the regeneration of the injured spinal cord tissue. This study examines the activation of Angiotensin II type 2 receptors (AT_2 receptors) and their involvement in the formation of new blood vessels, their integration into functional vasculature, and the evaluation of their overall impact on functional improvement. The pharmacological stimulation of the AT_2 receptor was conducted in an experimental model of severe spinal cord compression in adult female Wistar rats using the selective AT_2 receptor agonist CGP42112 (0.1 mg/kg per day) continuously administered by osmotic minipumps (s.c.). Within four weeks after trauma, the expression profile of the AT_2 receptor in the injured spinal cord correlated with the increased permeability of the blood-spinal cord barrier, as analysed through Evans blue tracing, and with the expression of vascular endothelial growth factor-A (VEGF-A). After 28 days following the trauma, our analyses using the qRT-PCR and ELISA showed an increase in the expression of proangiogenic markers including VEGF-A and its receptor, angiopoietin 1 and 2, and PECAM. This was observed after the stimulation of AT_2 receptors, compared to spinal cord injury alone. These results were also consistent with functional recovery. During the 28-day post-traumatic period, the motor function recovered rapidly, and the improvement was more significant after AT_2 receptor stimulation compared to spinal cord injury alone (BBB locomotor score: 10.4 points vs. 9 points). The improvement was strongly negatively correlated (Pearson r = -0.908) with a notably shorter latency (7.03 ms vs. 10.8 ms). Many of these positive effects were partially or completely prevented by blocking the AT_2 receptor. Our results indicate that the AT_2 receptors are likely involved in revascularization, and their activation may promote the proangiogenic signalling pathway in the injured spinal cord.

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Induced pluripotency and its potential in modeling of oncologic and neurodegenerative diseases

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Induced pluripotency is a state-of-the-art technique that allows the reprogramming of any fully differentiated somatic cell into stem cells. This technology, developed in 2006 by Japanese researcher Shinya Yamanaka and awarded with Nobel Prize in 2012 opened new fascinating horizons in the field of regenerative medicine as well as in the field of in vitro disease modeling. The research team of *Cellphie lab* at Jessenius Faculty of Medicine in Martin previously utilized this technique to prepare novel cell models or cell lines for modeling neurodegenerative diseases. The first Slovak induced pluripotent stem cell lines modeling sporadic form of amyotrophic lateral sclerosis and later Duchenne muscular dystrophy were already prepared by our team. The development of other cell lines modeling neurodegenerative diseases is currently in progress in our lab. Interestingly, we found that the reprogramming approach seems to be also applicable for the generation of special, 3D culture cancer cell lines from different types of tumors like neuroendocrine tumors, pancreatic ductal adenocarcinoma and colorectal cancer. Protocol designed and later modified by our team already led to the development of two unique cancer cell lines that were accepted by the American Tissue Culture Collection (ATCC) repository for general distribution to the researchers. Induced pluripotency and cell reprogramming towards a less differentiated state probably makes the hard-to-survive cancer cells isolated from tumor tissues more aggressive and more stem cell-like, thus promoting the cancer cells to be more effectively seeded on the plates. In conclusion, induced pluripotency is an extremely useful technique for the preparation of cell models in the field of neurology as well as oncology.

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Mesenchymal stem cells conditioned medium affects neurons and glial cells differently depending on the time of conditioning

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Mesenchymal stem cells (MSC) represent a source of bioactive substances capable of inducing the survival and regeneration of nerve cells. Recent studies with MSC conditioned medium (MSC-CM) have suggested the possibility of its use in regenerative medicine instead of whole cells. The aim of this in vitro study was to monitor the paracrine effect of rat bone marrow-derived mesenchymal stem cells (BMMSCs), using conditioned media prepared by conditioning BMMSCs for 24 hours (CM24), 48 hours (CM48), and 72 hours (CM72) in serum-free medium. We investigated the effect of these CMs on neurite length and density of DRG neurons and the proliferative activity of DRG glial cells . Proteomic profiles of individual secretomes were analyzed by mass spectrometry, and the concentrations of four selected neurotrophins (BDNF, NGF, GDNF, and VEGF) were determined using ELISA.

Proteomic analysis revealed an increased number of proteins in CM72, many of which are involved in neuroregenerative processes. ELISA documented a gradual increase in the concentration of two neurotrophins (NGF, VEGF), peaking at CM72. In vitro data suggest a different effect of BMMSC-CM on glial cell population and neurite outgrowth.

Our results indicate variability in the number and representation of identified specific proteins, concentrations of monitored neurotrophins, and the effect of CM on individual cell populations under in vitro conditions. Findings from the in vitro part of the study suggest that media obtained after long-term conditioning may have a more significant impact on the density/proliferation of glial cells, while media obtained from earlier conditioning times more significantly affected the neurite outgrowth and density.

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Neonatal nervous tissue exhibits superior reparative potential compared to the mature nervous tissue after minimal spinal cord injury

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Spinal cord injury (SCI) resulting from trauma decreases the quality of human life. Numerous clues indicate that the limited endogenous regenerative potential is a result of the interplay between the inhibitory nature of mature nervous tissue and the inflammatory actions of immune and glial cells. Knowledge gained from comparing regeneration in adult and juvenile animals could draw attention to factors that should be removed or added for effective therapy in adults. Therefore, we generated a minimal spinal cord injury (mSCI) model with a comparable impact on the spinal cord of Wistar rats during adulthood, preadolescence, and the neonatal period. The mechanism of injury is based on unilateral incision with a 20 gauge needle tip according to stereotaxic coordinates into the dorsal horn of the L4 lumbar spinal segment. The incision should harm a similar amount of gray matter on a coronal section in each group of experimental animals. According to our results, the impact causes mild injury with minimal adverse effects on the neurological functions of animals, but still has a remarkable effect on nervous tissue and its cellular and humoral components. Testing the mSCI model in adults, preadolescents, and neonates revealed a rather anti-inflammatory response of immune cells and astrocytes at the lesion site, as well as increased proliferation in the central canal lining in neonates compared with adult animals. Our results indicate that developing nervous tissue could possess superior reparative potential and confirmed the importance of comparative studies to advance in the field of neuroregeneration.

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Keywords: spinal cord injury, inflammation, development, glial scar, neural stem cells

Negative perception of a stressful situation is not associated with salivary concentrations of the stress hormone cortisol in toddlers

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A negative perception of stressful situations early in life may represent a greater risk for short-term and long-term negative outcomes including neurological and psychiatric disorders, throughout the lifespan. Stressor paradigms used in toddlers, such as a separation from a caregiver or exposure to a novel object or stranger have not proven an association with stress hormone concentrations. The present study aims to test the hypothesis that a negative perception of a different stressor correlates with salivary concentrations of the stress hormone cortisol during a still-face paradigm. The sample consisted of 41 toddlers (17 girls and 24 boys) aged 7 – 9 months accompanied by their mothers. The still-face paradigm consisted of three phases. During the first phase (3 min), the mother was playing with the baby. During the second phase (2 min) the mother looked at the baby with her face remaining still. This phase was followed by a reunion (3 min). The mother and her baby were left in the examination room alone while being recorded by 2 cameras. The saliva for cortisol measurements was collected before the task and 20 min thereafter. The baby's negative perception of the situation was evaluated from the videos as the time spent crying and the latency to cry using the program H77+. The results failed to confirm the association between cortisol concentrations and signs of negative stress perception measured. Numerically, the boys started to cry earlier and spent more time crying than girls, however, the difference failed to reach statistical significance. The latter finding is supportive of the suggestion that early life stress puts males at an increased risk of presenting with neurodevelopmental disorders.

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A model for studying long distance axonal regeneration in the rat

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In clinical practice, the prognosis after segmental injuries to the peripheral nerve with gaps longer than 3cm is unfavorable. It is known that regenerating axons are capable of growing for long distances within the preserved distal stump. It is not clear why the growth fails within long grafts or graft alternatives after nerve reconstruction.

For understanding this paradox, a model that allows removal of long segments of the peripheral nerve is necessary. This can be obviously made in large animal models, but these are expensive and laborious. In our study, we document that clinically relevant segmental injuries can be made in small laboratory animal, as well. In the rat, tail nerves are the longest peripheral nerves in their body. We suggest that ventral caudal nerve (VCN) may serve as a model for studying segmental nerve injuries and long distance regeneration.

For this purpose, we have studied the anatomy and morphometry of the VCN in control animals. 10 cm long segment of the VCN was removed, and transversal sections were collected at 10 mm distances. The myelinated axons were counted, and the series of data were used to characterize the craniocaudal tapering of the nerve. In a separate group of animals, retrograde tracing with Fluorogold was used to localize and quantitate the spinal neurons projecting their axons into the VCN.

After complete nerve transection, the time course of histopathological changes in the distal segment was studied. The primary goal was to define the time needed for axonal disintegration. In later periods, axonal debris removal and rearrangement of tissue elements was documented.

After compression injury (axonotmesis), Wallerian degeneration was followed by spontaneous regeneration of axons. We show that the growing axons will span the 10 cm distance within 4-8 weeks. After different survival periods, the numbers of regenerating axons were counted at 10mm distances. These data were used to characterize the dynamics of axonal regeneration during 4 months' survival period. In the present study we show that axonal regeneration across 10 cm distance can be studied and quantitatively analyzed in a small laboratory animal.

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Heart Rate Variability in evaluation of autonomic dysfunction in idiopathic REM-sleep behaviour disorder

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Introduction: Nearly 80% of people diagnosed with idiopathic REM sleep behaviour disorder (iRBD) via video-polysomnography (v-PSG) are expected to be in the prodromal stage of an alpha-synucleinopathy. Signs of autonomic dysfunction can appear earlier than motor or cognitive alpha-synucleinopathy symptoms. Heart Rate Variability (HRV) can potentially be an objective measurement of autonomic dysfunction, and furthermore can be obtained directly from v-PSG.

Aim: The aim of this study was to evaluate dysautonomia in iRBD subjects using HRV obtained during different sleep stages and wakefulness from v-PSG.

Methods: Subjects positively screened by an RBD screening questionnaire (RBD-SQ) underwent v-PSG to diagnose RBD. HRV obtained from v-PSG recordings was correlated to dysautonomia evaluated from a Non-Motor Symptoms Scale (NMSS) questionnaire. Optimal cut-off values of HRV parameters to predict dysautonomia were calculated using receiver operating characteristics (ROC) - area under the curve (AUC) analysis. The effect of confounder variables was predicted with binomial logistic regression and multiple regression analyses.

Results: Out of 72 positively screened subjects, 29 subjects were diagnosed as iRBD (mean age 66 ± 7.7 years) by v-PSG. Eighty-three per cent of the iRBD subjects in our cohort were at the time of diagnosis classified as having possible or probable prodromal Parkinson's Disease (pPD) compared to zero subjects being positively screened in the control group. The iRBD-positive subjects showed significant inverse correlations of NMSS score, particularly to log low-frequency (LF) component of HRV during wakefulness: $r = -0.59$ ($p = 0.001$). Based on ROC analysis and correlation between NMSS score, log LF during wakefulness (AUC 0.74, cut-off 4.69, sensitivity 91.7%, specificity 64.7%, $p = 0.028$) was considered as the most accurate predictor of dysautonomia in the iRBD group. Apnoea-hypopnoea index (AHI) negatively predicted dysautonomia in the iRBD group. None of the HRV components was able to predict the presence of iRBD in the full cohort. Age, gender, and PSG variables were significant confounders of HRV prediction.

Conclusion: The presented study did not confirm the possibility of using HRV from v-PSG records of patients with iRBD to predict dysautonomia expressed by questionnaire methods. This is probably due to several confounding factors capable of influencing HRV in such a cohort.

Key words: RBD Screening Questionnaire; dysautonomia; heart rate variability; idiopathic REM sleep behaviour disorder.

The role of sphingosine-1-phosphate receptor modulator (Siponimod) in spinal cord injury

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Spinal cord injury (SCI) is a pathological condition associated with excessive activation of microglia that leads to the overproduction of proinflammatory cytokines and causes neuronal injury. The molecular mechanisms of this process are still not fully elucidated and therapies for SCI are marginally effective. Sphingolipids are bioactive lipids that represent an interesting therapeutic target for SCI. Sphingosine-1-phosphate (S1P) is a sphingolipid generated from sphingosine by sphingosine kinases (SPHK1 and -2) that plays an important role in mediating inflammation, cell proliferation and survival.

This study aimed to examine the mRNA levels of enzymes and receptors engaged in S1P signalling as well as pro-survival sirtuin 1 (SIRT1) in the cerebellum and spinal cord (SC) of rats after SCI. Moreover, the effect of Siponimod (SIPO, S1P receptor (S1PR) 1&5 modulator) was evaluated.

Female Wistar Rats (weight 300g±30g) were used in the experiment and divided into the three experimental groups: Control, Compressed (COMP) and Compressed rats that received SIPO (*i.p.* 1mg/kg). Control and COMP animals received only vehicle (DMSO). SCI was induced by compression at Th9 vertebral level. The spinal cord was exposed very carefully and compressed using a device with a weight of 40g for 15 minutes. SIPO was administered 30 minutes after the compression. After 7 days of SIPO administration cerebellum and cranial segment (above site of injury) of SC was isolated for qPCR analysis. All experiments were analysed using one-way ANOVA followed by Tukey's post-hoc test (n=4-6 rats/group).

We observed a significant reduction of *Sphk2*, *S1pr1*,*-3*,*-5* and *Sirt1* gene expression in both cerebellum and SC of COMP rats. The *Sphk1* mRNA levels remained unchanged in both analysed structures. SIPO administration further downregulated *Sphk2* mRNA levels in cerebellum and upregulated its expression in SC. Moreover, SIPO showed a tendency to elevate mRNA levels of *S1pr1* in SC and *S1pr5* in both structures.

These results indicate that genes encoding S1P signalling proteins and SIRT1 are altered after SCI. We also showed that SIPO acts differently on Sphk2 expression and this effect is structure/region-dependent. Obtained results suggest that modulation of S1P receptors can be considered as a potentially important factor in the pathology of SCI.

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Bridging the Gap: P2X7 Receptor as a Molecular Link Between α-Synuclein Toxicity, Parkin Dysfunction, and Mitochondrial Impairment

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While both α-Synuclein (α-Syn) and Parkin abnormalities and their associated dysfunctions are implicated in the molecular pathways of Parkinson's disease (PD), in this study, we investigated functional relationships between these two proteins using various *in vitro* and *in vivo* models. Our findings reveal a direct connection between the downregulation of Parkin and extracellular α-Syn signaling. Our in vitro study showed that α-Syn-induced oxidative/nitrosative stress plays a crucial role in the S-nitrosylation of Parkin, leading to its impaired function and degradation. Also in the murine model of α-synucleinopathy, the progressive decrease in Parkin protein was observed in the midbrain. Furthermore, the reduction in Parkin levels triggered by α-Syn treatment largely depended on activating the purinergic P2X7 receptor. Extracellular α-Syn – evoked P2X7 receptor activation resulted in mitochondrial impairment and the decrease of Parkin level, ultimately causing the loss of Parkin-mediated mitophagy and accumulation of the dysfunctional mitochondria. Conversely, elevating Parkin levels prevented mitochondrial dysfunction and oxidative stress induced by exogenous α-Syn. These findings provide compelling evidence for the direct association of P2X7 receptors activation, and Parkin dysfunction with extracellular α-Syn, highlighting its critical role in the pathophysiology of PD.

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Mechanism of Selective Neurodegeneration after Global Brain Ischemia. Proteasomal versus Endoplasmic Reticulum Stress

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A brief period of transient global brain ischemia leads to selective ischemic neurodegeneration associated with death of hippocampal CA1 pyramidal neurons days after reperfusion. The mechanism of such selective and delayed neurodegeneration is still uncertain.

Our work aimed to study the involvement of proteasomal and endoplasmic reticulum (ER) stress in ischemic neurodegeneration. We have performed laser scanning confocal microscopy analysis of brain slices from control and experimental animals that underwent global brain ischemia for 15 minutes and varying times of reperfusion. We have focused on PUMA, a proapoptotic protein of the Bcl-2 family overexpressed in response to both proteasomal and ER stress, and p53, which controls expression of PUMA. We have also examined the expression of HRD1, an E3 ubiquitin ligase that was shown to be overexpressed after ER stress. We have also examined potential crosstalk between proteasomal and ER stress using cellular models of both proteasomal and ER stress.

We demonstrate that global brain ischemia is associated with an appearance of distinct immunoreactivity of PUMA and p53 in pyramidal neurons of the CA1 layer of the hippocampus 72 hours after ischemic insults. Such changes correlate with a delay and selectivity of ischemic neurodegeneration. Immunoreactivity of HRD1 observed in all investigated regions of rat brain was transiently absent in both CA1 and CA3 pyramidal neurones 24 hours after ischemia in the *hippocampus,* which does not correlate with a delay and selectivity of ischemic neurodegeneration. We do not document significant crosstalk between proteasomal and ER stress.

Our results favour dysfunction of the ubiquitin proteasome system and consequent p53-induced expression of PUMA as the main mechanisms responsible for selective and delayed degeneration of pyramidal neurons of the hippocampal CA1 layer in response to global brain ischemia.

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Effect of early chronic stress on proliferation and migration of neuroblasts in the rat rostral migratory stream

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Postnatal neurogenesis in the subventricular zone (SVZ) – rostral migratory stream (RMS) –olfactory bulb (OB) is a complex process that involves different phases of neuronal maturation: proliferation, migration, differentiation of progenitor cells, and integration of new neurons into existing neuronal circuits. It has been found that individual processes of neurogenesis in the SVZ-RMS-OB can be influenced by environmental factors such as different types of radiation, stress, enriched environment, etc. The aim of this study was to investigate the effect of chronic early stress induced by separation of rat pups from the mother during the first three postnatal weeks (daily for 180 min.) on proliferation and migration of neuronal precursors in the RMS. Pups exposed to separation were allowed to survive until adulthood. To assess cell proliferation, brain sections were processed for immunohistochemistry, using Ki-67 antibody against an endogenous marker of proliferation. Proliferating cells were counted by Disector software in three anatomical parts of the RMS, the vertical arm, the elbow and the horizontal arm. For analyzing neuroblast migration, the RMS was dissected out of the brain sections, and the RMS explants were cultured *in vitro* for 72 hours. Then, the explants were processed for immunohistochemistry using antibody against neuroblasts (anti-Dcx). Neuroblast migration was quantified by measuring the area of migration and longest migration length for each explant using ImageJ software. We found that chronic early stress caused by maternal separation resulted in significant decrease in cell proliferation in all parts of the RMS of adult rats in comparison with control animals. Quantitative analysis of neuroblast migration based on explants methodology showed non-significant increase in both parameters, the area of migration and longest migration length in group of animals that underwent chronic early stress. Furthermore, in this group of rats, the neuroblasts of the RMS explants tended to migrate individually, rather than in chains, in contrast to control animals. We can conclude that chronic early stress caused by maternal separation had an impact on neurogenic processes such as cell proliferation and migration in the RMS of adult animals.

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PPAR-α synthetic ligands and their effect on the level of mRNA expression related to redox state and mitochondria proteins in different parts of the brain in an animal model of Alzheimer's disease

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Peroxisome proliferator –activated receptor α (PPAR-α) is a ligand activated transcription factor. Recently genome–wide association studies and several novel data indicating promising neuroprotective effect of PPAR-α.The last results suggest that PPAR-α pharmacological ligands could be repurposed for treatment of Alzheimer's disease (AD) and other neurological and psychiatric disorders.

Our study aimed to analyze the effect of PPAR-α synthetic ligands: Fenofibrate (FF) and GW7647 on transcription of genes involved in amyloid β metabolism and mitochondria function in different parts of the brain in an animal AD model.

The studies were carried out using familiar AD transgenic mice with" London" mutation (V717I) of 3 and 12 month old treated with GW 7647 s.c. in a dose of 5mg/kg body weight (bw) or with Fenofibrate (FF) i.p. in a dose of 30mg/kg bw during 14 days. Control animals were treated with appropriate solvents respectively. The brain cortex and hippocampus were used for analysis. Quantitative qPCR and immunochemical, biochemical methods were applied.

The role of PPAR-α synthetic ligands in genes expression was evaluated on mRNA and protein levels in the brain parts of 3 and 12 month old AD Tg mice. Our study indicated that transcription of genes encoded enzymes involved in free radical homeostasis (such as *Sod2*, *Sirt1* and *Parp1*) was significantly decreased in the brain cortex of 3-month-old AD Tg mice. Moreover, our last data showed that expression of genes coding proteins of mitochondria biogenesis (*Ppara, Ppargc1a, Nrf2* and *Tfam*) were down regulated in the brain cortex of 12-month-old AD Tg mice. GW7647 enhances the expression of genes related to mitochondria biogenesis as compared to control mice (without transgene). However, the current study demonstrated that in the hippocampus GW7647 decreased the level of mRNA genes engaged in mitochondria biogenesis and likely affected their turnover as well. It is suggested that GW7647 may affect brain cortex and hippocampus differently. In the following studies, the effect of FF in AD Tg mice was investigated and our data revealed that FF activates selected genes involved in mitochondria dynamic (*Mfn1*, *Opa1*) and also enhances the transcription of *Nrf2, Tfam,* involved in mitochondria biogenesis in the brain cortex of 12 month old AD Tg mice. Moreover, FF influences expression of selected genes related to Aβ metabolism (decreases *Bace1*, *Psen2 and* activates *Adam10*, *Ide)* in the brain cortex of AD Tg mice. It is suggest that FF may exert effect on Aβ homeostasis in the brain of AD Tg mice.

Our results indicate novel aspects of the molecular mechanism of PPAR-α agonist's action, which may depend on the brain parts and age of AD Tg mice. However, further studies are necessary to better understand the role of PPAR-α ligands in AD.

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Current approach in thrombolytic therapy research – how to break the translational block

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In vivo preclinical research on thrombolytic therapy of stroke is complicated in terms that the thrombolysis efficacy and the thrombolysis treatment safety cannot be evaluated simultaneously. Therefore, we developed two models for testing new thrombolytics and new dual treatment methods.

The thrombolysis "efficacy model" is performed with continuous X-ray scanning of artificial clots embolized into the systemic circulation in rats. Artificial clots are human fibrin based and barium labelled. The data measured are the area under the thrombolytic curve (%*min), the lysis rate (%/min) and the relative change in clot area (%) over time. The model is time-efficient with data amplification, because three artificial clots are administered per rat. The model has zero mortality (0 from 174) and in animal including to study is 100% success rate (174 from 174) in minimally one clot lysis tracking/rat.

The safety study model is based on "classic" thromboembolic Middle Cerebral Artery Occlusion and uses the same type of clot as the "efficacy model". However, accepted is all embolization to the large cerebral vessels (anterior, middle, or posterior cerebral artery). Thrombolysis is initiated four hours after stroke induction. After 24 hours, the brain is impregnated for micro computed tomography (miCT) examination and haemorrhagic transformation is detected and quantified. Brain sections can be further processed using topographic mass spectrometry (LA-ICP-MS) and histology. Ex-vivo miCT scan allows repetitive image analysis in post scanning time in different type picture analysis or re-analysis in new setup (e.g., prospectively with the help of artificial intelligence). We have repeatedly used the developed models to test novel thrombolytics or thrombolytic procedures and to compare them with standard alteplase and/or tenecteplase stroke treatments.

The presented novel approach for thrombolytics treatment testing in stroke with two complementary models allows for streamlining preclinical research by reducing the number of animals used, almost 100% inclusion of experimental subjects for data processing, and subsequent reanalysis of digital data using artificial intelligence, if beneficial.

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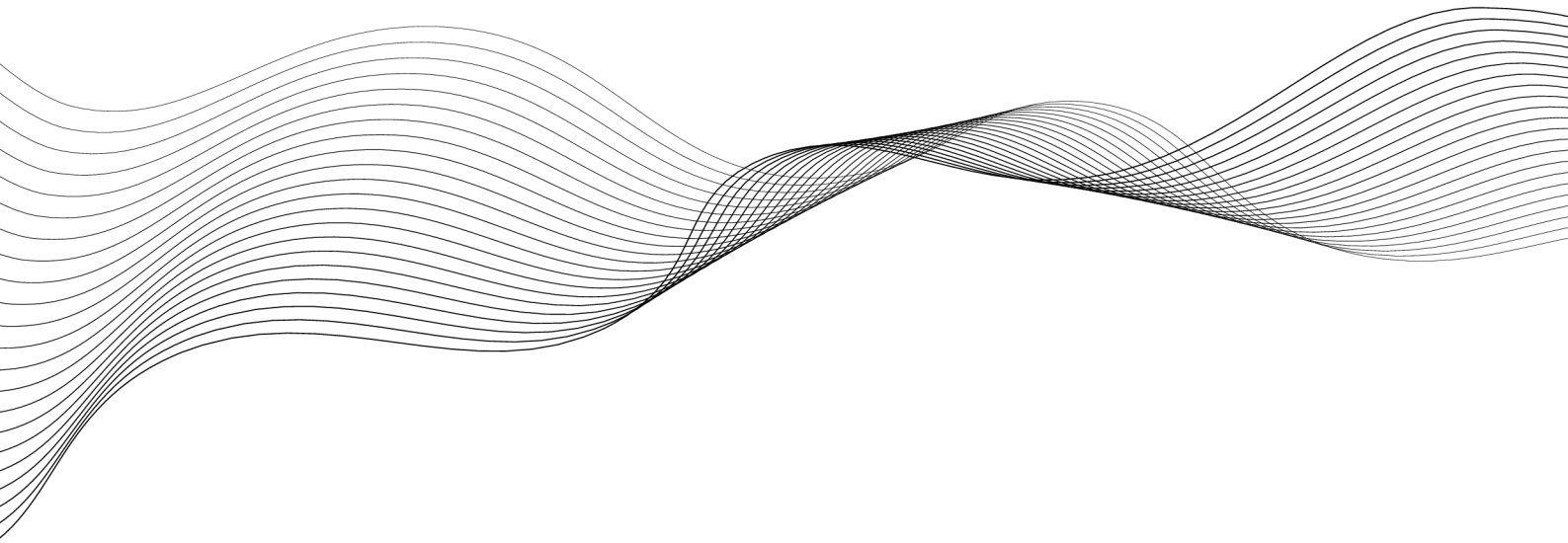
NU23-08-00499 Improving the Efficacy and Safety of Cerebral Infarction Recanalization Using Dual Thrombolytic Therapy.



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