Croatian neuroscience congress

24th and 25th September 2021
virtual congress

CROATIAN
SOCIETY FOR
NEUROSCIENCE

8. CROATIAN NEUROSCIENCE CONGRESS

ORGANIZERS

Croatian Society for Neuroscience (CSFN)

Croatian Institute for Brain Research (CIBR)

Centre of Excellence for Basic, Clinical and Translational Neuroscience

School of Medicine, University of Zagreb

Croatian Academy of Sciences and Arts, Department of Medical Sciences

ORGANIZING COMMITTEE

Marijan Klarica, Miloš Judaš, Svjetlana Kalanj Bognar, Mario Vukšić, Zoran Đogaš, Darko Chudy, Marija Heffer, Ivica Kostović, Lipa Čičin Šain, Željka Krsnik, Nataša Jovanov Milošević, Goran Šimić, Goran Sedmak, Pero Hrabač, Tena Popović, Mihaela Bobić-Rasonja, Dinko Smilović

SCIENTIFIC COMMITTEE

Ivica Kostović (Head of the committee), Vida Demarin, Norman Sartorius, Nenad Šestan, Paško Rakić, Mladen Roko-Rašin, Ante Padjen, Maja Valić, Zoran Đogaš, Ivana Munitić, Rozi Andretić Waldovski, Marija Heffer, Nataša Šimić, Marina Vidaković, Zdravko Petanjek, Srećko Gajović, Dinko Mitrečić, Zdravka Poljaković, Milan Radoš

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24th and 25th September 2021
Zagreb - Virtual congress

GENERAL INFORMATION

SCIENTIFIC PROGRAMME OVERVIEW

Presidential lecture

***Speaker*:** Ivica Kostović (Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia)

Special symposium „Neuroinflammation and COVID-19“

***Speakers*:** Marija Santini (University Hospital for Infectious Diseases “Dr Fran Mihaljević”, Zagreb, Croatia); Mario Habek (Department for Neurology, University Hospital Centre Zagreb, Croatia); Milan Radoš (School of Medicine, University of Zagreb, Croatia); Ilija Brizić (School of Medicine, University of Rijeka, Croatia)

Presidential symposium „Current trends in neuroscience“

***Speakers*:** Dean Nižetić (Queen Mary University of London, United Kingdom); Dimitri Krainc (Northwestern University, Chicago, United States); Lana Vasung (Harvard Medical School, Boston, United States); Stephan Schwarzacher (Goethe University, Frankfurt am Main, Germany)

Thematic symposia

***Speakers*:**

Symposium I. “Sleep and sleep research during the COVID-19 pandemic” - Zoran Đogaš (School of Medicine, University of Split, Croatia); Rozi Andretić Waldowski (Department for Biotechnology, University of Rijeka, Croatia); Ivana Rosenzweig (King’s College, London, United Kingdom); Ivana Dodig Pavlinac (School of Medicine, University of Split, Croatia)

Symposium II. “Brain lipidomics” - Marija Heffer (School of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia); Željka Korade (University of Nebraska Medical Center, Omaha, United States); Karoly Mirnics (University of Nebraska Medical Center,

Omaha, United States); Kristina Mlinac-Jerković (School of Medicine, University of Zagreb, Croatia)

Symposium III. "Alzheimer's disease" - Maja Jazvinšćak Jembrek (Ruđer Bošković Institute, Croatia); Petra Kalember (Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia); Mirjana Babić Leko (School of Medicine, University of Split, Croatia); Jelena Osmanović Barilar (School of Medicine, University of Zagreb, Croatia); Tena Sučić Radovanović (School of Medicine, University of Zagreb, Croatia); Dubravka Švob Štrac (Ruđer Bošković Institute, Croatia)

Symposium IV. "Preclinical models of neurological diseases" - Dinko Mitrečić (School of Medicine, University of Zagreb, Croatia); Miranda Mladinić Pejatović (Department for Biotechnology, University of Rijeka, Croatia); Marta Balog (School of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia); Marina Dobrivojević Radmilović (School of Medicine, University of Zagreb, Croatia); Damir Sapunar (School of Medicine, University of Split, Croatia); Srećko Gajović (School of Medicine, University of Zagreb, Croatia)

Poster session topics

Basic neuroscience; Clinical neuroscience; Cognitive neuroscience; Hypoxic-ischemic damage; Molecular neuroscience; Neurodegenerative disorders; Neurodevelopmental basis of cognitive, mental and neurological disorders; Sleep

Satellite events

- Presentation of the Centre of Excellence for Basic, School of Medicine University of Zagreb, Clinical and Translational Neuroscience, CoreNeuro (Miloš Judaš and collaborators)
- Students in Neuroscience (Student section for neuroscience)

POSTER SESSIONS

Posters will be displayed during two on-line Poster sessions on Saturday, 25th September 2021 according to a schedule announced at the congress web-site. Presence of presenting authors is mandatory during specified scheduled poster session. Presenting authors of posters will be obliged to present the main findings of their work in 3 minutes talk/on-line presentation,

according to a schedule. Poster session I. will be held on-line from 9.30 to 12.00 and will cover following topics: Basic neuroscience, Molecular neuroscience, Cognitive neuroscience, Neurodevelopmental basis of cognitive, mental and neurological disorders, and Sleep. Poster session II. will take place from 12.15 to 14.30 and will present topics related to: Clinical neuroscience, Hypoxic-ischemic damage, and Neurodegenerative disorders.

POSTER PRESENTATION AWARD

Poster presentations are important scientific contributions, therefore a prize for the best poster presentations is established. In both poster sessions, the best and the second-best poster will be awarded.. The selection will be done on the basis of scientific merit and clarity of presentation as judged by high-ranking board made up from three members of the Programme Committee. The awards will be announced during the farewell speech.

LANGUAGE OF THE CONFERENCE

The official language of the meeting is English and Croatian.

REGISTRATION FEES

	Registration deadline September, 3rd 2021
CSfN member	500
Non-member of CSfN	750
PhD student	250
Graduate student	waived

***Fee prices are given in Croatian kunas.

Included in the registration fee:

Unlimited access to all on-line sessions and events during the period of the meeting; access to all poster sessions; Certificate of attendance.

PROGRAMME

Friday, 24th September 2021

10.00-10.15 Welcome and opening remarks by President of the Croatian Society for Neuroscience - **Marijan Klarica**

Welcome video-message from Secretary General of the Federation of European Neuroscience Societies - **Dóra Reglody**

10.15-11.00 **PRESIDENTIAL LECTURE**

Introductory words - Marijan Klarica and Miloš Judaš (Chairs)

Ivica Kostović: Subplate compartment - main playground for construction and functions of the human fetal cortex

11.00 **SYMPOSIUM "NEUROINFLAMMATION AND COVID-19"**

Introductory words - Zoran Đogaš and Maja Valić (Chairs)

11.15-11.35 **Marija Santini**: COVID-19 and encephalitis

11.40-12.00 **Milan Radoš**: The spectrum of neuroimaging findings on MRI in patients with COVID-19

12.05-12.25 **Ilija Brizić**: Mechanisms of neuroinflammation and pathology induced by virus infection

12.30-12.50 **Mario Habek**: COVID-19 in people with multiple sclerosis: what have we learned so far?

12.50-13.00 *On-line discussion*

13.15 **PRESIDENTIAL SYMPOSIUM "CURRENT TRENDS IN NEUROSCIENCE"** *Introductory words - Marijan Klarica and Miloš Judaš (Chairs)*

13.30-13.50 **Dean Nižetić**: Dissecting pathogenic and protective mechanisms for Alzheimer's disease using human cerebral organoid models

13.55-14.15 **Stephan Schwarzacher**: Synaptic plasticity in postnatal and adult hippocampal neurogenesis

14.20-14.40 **Dimitri Krainc**: The interplay of mitochondrial and lysosomal dysfunction in Parkinson's disease

14.45-15.05 **Lana Vasung**: Kinematic study of *in utero* fetal movements

15.05-15.15 *On-line discussion*

THEMATIC SYMPOSIA

16.00-17.30 I. SLEEP AND SLEEP RESEARCH DURING THE COVID-19 PANDEMIC

Introductory words - Zoran Đogaš and Renata Pecotić (Chairs)

Zoran Đogaš: Sleep and sleep research update in the COVID-19 pandemic

Rozi Andretić Waldowski: Back to the basics - learning how redox modulation regulates sleep and addiction using animal model

Ivana Rosenzweig: Effects of lockdown during the COVID-19 pandemic on sleep and mental health

Ivana Pavlinac Dodig: Sleep and lifestyle habits during the COVID-19 pandemics in Croatia

On-line discussion

16.00-17.30 II. BRAIN LIPIDOMICS

Introductory words – Marija Heffer and Svjetlana Kalanj Bognar (Chairs)

Marija Heffer - Lipidomic analysis of demyelination associated with complex ganglioside deficiency

Željka Korade - Disruption of brain sterol biosynthesis by commonly used prescription medications

Karoly Mirnics - Interaction of genetics, pregnancy and medications on the developing brain

Kristina Mlinac Jerković - Gangliosides framework for optimal function of plasma membrane ion transporters

On-line discussion

18.00-19.30 III. ALZHEIMER'S DISEASE

Introductory words – Goran Šimić (Chair)

Maja Jazvinšćak Jembrek - Pro-oxidative effects of flavonols in copper-induced oxidative stress

Petra Kalember - The role of proton magnetic resonance spectroscopy in diagnostics and investigating the disease course and treatment response in Alzheimer's disease

Mirjana Babić Leko - *APOE* genotype and essential metals in Alzheimer's disease

Jelena Osmanović-Barilar - Different effects of acute central inhibition of the GLP-1 and glucose-dependent insulinotropic peptide receptors in rat model of sporadic Alzheimer's disease

Tena Sučić Radovanović - The role of arterial spin labeling magnetic resonance imaging technique in diagnostics of Alzheimer's disease: our initial findings

Dubravka Švob Štrac - Dehydroepiandrosterone and brain-derived neurotrophic factor: potential therapeutic targets in Alzheimer's disease

On-line discussion

18.00-19.30 IV. PRECLINICAL MODELS OF NEUROLOGICAL DISEASES

Introductory words – Srećko Gajović (Chair)

Dinko Mitrečić - Application of stem cells in brain diseases: focus on mitochondria and cell death

Miranda Mladinić Pejatović - Opossum Monodelphis domestica in research of neuroregeneration and neurodegeneration

Marta Balog - Rat model of chronic stress: connection to neurodegeneration

Marina Dobrivojević Radmilović - Retinal ischemia in rodent stroke models

Damir Sapunar - What can rats tell us about neuropathic pain?

Srećko Gajović - Repairing the mouse brain after stroke

On-line discussion

Saturday, 25th September 2021

9.30-12.00	POSTERS AND DISCUSSIONS I
12.15-14.30	POSTERS AND DISCUSSIONS II
14.30-15.00	**Presentation of Centre of Excellence for Basic, Clinical and Translational Neuroscience, School of Medicine University of Zagreb** Miloš Judaš and collaborators
15.00 – 15.45	**Students in neuroscience** *Introductory words – Dinko Smilović and Goran Sedmak (Chairs)* Activity and history of the student section of neuroscience & Student journal Gyrus – short movies
16.00	**Conference closing remarks and announcement of the Best poster award**

POSTER PRESENTATIONS

Saturday, 25th September 2021 - POSTER SESSION I.

9.30 – 12.00

P1 BASIC NEUROSCIENCE

P2 MOLECULAR NEUROSCIENCE

P3 COGNITIVE NEUROSCIENCE

P4 NEURODEVELOPMENTAL BASIS OF COGNITIVE, MENTAL AND NEUROLOGICAL DISORDERS

P5 SLEEP

Saturday, 25th September 2021 - POSTER SESSION II.

12.15 – 14.30

P6 CLINICAL NEUROSCIENCE

P7 HYPOXIC-ISCHEMIC DAMAGE

P8 NEURODEGENERATIVE DISORDERS

LIST OF POSTERS

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PP1

OPTOELECTRONIC INDUCTION OF FIELD EXCITATORY POSTSYNAPTIC POTENTIAL BY ORGANIC PHOTOCAPACITOR

Ivan Strinić, Aleksandar Opančar, Andrea Blažević, Anja Mioković, Vedran Đerek, Nikola Habek

PP2

ROSTRO-CAUDAL DIFFERENCES IN THE RATIO OF GABAERGIC NEURONS SUBTYPES THROUGH THE RAT NEOCORTEX

Andrea Blažević, Ivan Banovac, Dora Sedmak, Ana Hladnik, Zdravko Petanjek

PP3

MALE WISTAR RATS RAPIDLY HABITUATE TO CAT ODOUR PREDATOR STRESS TEST FOR ANXIETY

Ante Tvrdeić, Ljiljana Poljak, Alen Babacanli, Branko Miše

PP4

GRANULE CELLS EXHIBIT REDUCED SPINE DENSITY AND HOMEOSTATIC CHANGES OF DENDRITIC SPINE PARAMETERS IN THE DENTATE GYRUS OF MICE LACKING TUMOR NECROSIS FACTOR (TNF)

Dinko Smilović, Michael Rietsche, Thomas Deller, Mario Vukšić

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TRANSCRIPTION FACTORS IN THE DEVELOPING HUMAN SUBTHALAMIC NUCLEUS

Ema Bokulić, Tila Medenica, Mihaela Bobić-Rasonja, Marija Milković-Periša, Nataša Jovanov-Milošević, Goran Sedmak

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A COMPARATIVE ANALYSIS REVEALS INTER-SPECIES PHENOTYPIC DIFFERENCES IN THE ADULT MAMMALIAN SUBTHALAMIC NUCLEUS

Tila Medenica, Ema Bokulić, Goran Sedmak

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TRANSIENT ROLE OF THE SUBPLATE NEURONS IN DIFFERENTIAL INGROWTH OF PULVINOCORTICAL AND GENICULOCORTICAL AXONS INTO THE PROSPECTIVE STRIATE AND EXTRASTRIATE CORTEX OF THE HUMAN FETAL BRAIN

Iris Žunić Išasegi, Janja Kopić, Dinko Smilović, Željka Krsnik, Ivica Kostović

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VENTRO-DORSAL DIFFERENCES IN PROPORTIONS OF GABAERGIC INTERNEURON POPULATIONS IN THE HUMAN PREFRONTAL CORTEX

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REGULATION OF BROWN ADIPOSE TISSUE ACTIVITY BY BRAIN UROGUANYLIN

Habek Nikola, Ratko Martina, Kordić Milan, Dugandžić Aleksandra

P2 MOLECULAR NEUROSCIENCE

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Mirjana Babić Leko, Matea Nikolac Perković, Gordana Nedić Erjavec, Nataša Klepac, Dubravka Švob Štrac, Fran Borovečki, Nela Pivac, Patrick R. Hof, Goran Šimić

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Barbara Vuić, Matea Nikolac Perković, Gordana Nedić Erjavec, Lucija Tudor, Marcela Konjevod, Nela Pivac, Dubravka Švob Štrac

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Ljiljana Poljak, Mirta Boban, John R. Falck, Marija Renić

ABSTRACTS

PRESIDENTIAL LECTURE

Subplate compartment - main playground for construction and functions of the human fetal cortex

Ivica Kostović
Professor Emeritus, Full Member of Croatian Academy of Sciences and Arts; Croatian Institute for Brain Research, Honorary Director; School of Medicine, University of Zagreb

This presentation discloses autobiographic overview of discovery and research on the subplate, the most prominent transient synapse containing compartment (SPC) of the human fetal cortex. The long-term study (49 years) of the histogenetic role of SPC revealed that it provides interactive milieu and capacity for guidance, sorting, "waiting" and target selection of thalamocortical and cortico-cortical pathways. The functional role of SPC during important transitional period in preterm infants is discussed in terms of sensory driven, spontaneous and evoked, and silent versus active synapses. The novel concept of tangential SPC nexus before cortico-cortical connectivity is established was formulated during the last few years. New vistas for role of SPC in the developing human cortex are now opened through new collaborative research on molecular mechanisms and translational approach in studies of origin of major neurodevelopmental disorders.

SYMPOSIUM “Neuroinflammation and COVID-19”

COVID-19 and encephalitis

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Coronaviral infections were associated with neurological manifestations even throughout previous outbreaks in the 20th century, during SARS (2002) and MERS (2012). Very soon, the clinical presentation of COVID-19 demonstrated that it affects not only the respiratory system but also blood vessels endothelium, producing numerous systemic and neurological manifestations. Neurological manifestations include myalgia, smell and taste disorders, encephalopathy, encephalitis, polyneuritis and stroke. These manifestations occur not only in acute COVID-19 but in the post or long-COVID-19 period too. The literature describing neurological manifestations of COVID-19 is abundant, but still, there is a lot to be revealed regarding the effectiveness of antiviral agents, immunomodulation and anticoagulant drugs. Glucocorticoids seem to be critical "game-changers" in the treatment of COVID-19. Yet, we are still far from knowing the effect of monoclonal antibodies, plasmapheresis or intravenous immunoglobulins on neurological manifestations of COVID-19. The same problem exists regarding the lack of knowledge of the thromboembolic prophylaxis impact on the incidence of stroke in COVID-19.

The spectrum of neuroimaging findings on MRI in patients with COVID-19

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In most patients with COVID-19, the clinical picture is mild and limited to the respiratory system, so neuroimaging is unnecessary. However, in patients who develop a more severe clinical picture, other organ systems, including the central nervous system, are often affected. Three primary pathophysiological mechanisms are thought to lead to brain damage in patients with COVID-19. The first is an overreaction of the immune system, the second is a disorder of the blood coagulation system, and the third is due to the direct invasion of the virus into brain cells. The spectrum of neuroimaging findings in patients with COVID-19 ranges from discrete lesions without special clinical significance to a severe, life-threatening condition. Numerous studies show that ischemic cerebrovascular stroke is the most common of all structural lesions, followed by intracerebral haemorrhage, venous thrombosis, and posterior reversible encephalopathy. In addition to the listed vascular disorders, inflammatory brain lesions as acute haemorrhagic necrotizing encephalitis, acute disseminated encephalitis or Miller-Fisher syndrome may occur less frequently. The most common neurological dysfunction in patients with COVID-19 is anosmia. However, although some studies claim that olfactory neuritis can be diagnosed on coronary MR FLAIR sections, this method does not appear to be reliable in distinguishing patients with anosmia from those with a sustained sense of smell. It should be noted that many patients complain of problems with concentration and memory after overcoming COVID-19. Although MR is a potent diagnostic tool for detecting structural brain damage, these functional cognitive disorders cannot be visualized by standard MR exam.

Mechanisms of neuroinflammation and pathology induced by virus infection

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Congenital cytomegalovirus (cCMV) infection is a leading viral cause of mental retardation and sensorineural hearing loss in infants and children. We have previously shown that infection of newborn mice with mouse CMV recapitulates major hallmarks of human cCMV infection: virus dissemination to the brain parenchyma, neuroinflammation, altered brain development, and sensorineural hearing loss. Early innate immune mediators, including NK/ILC1 cells, IFNγ, and TNFα, drive neurodevelopmental pathology, while adaptive T cells establish virus control and clearance of the infectious virus. Since latent CMV can reactivate at any time, the proinflammatory environment in the brain is maintained for life. Single-cell transcriptomic analysis of microglia and astrocytes from latently infected mice revealed that latent CMV infection drastically changes the composition of microglia at the single-cell level. At the same time, astrocyte homeostasis is minimally affected, indicating differential homeostatic features of these glial cells following infection. Microglial subpopulations associated with CMV latency have highly expressed genes encoding for MHC I and II molecules, and genes involved in response to interferon type I and II (Cxcl9, Cxcl10). These changes were not due to virus latency in microglia, since we did not detect viral genomes in these cells. Altogether, our results show that latent CMV infection in the brain leads to persistent neuroinflammation. Finally, lessons learned from studies of CMV infection in the brain in the context of CNS manifestation of SARS-CoV-2 infection will be discussed.

COVID-19 in people with MS: what have we learned so far?

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Multiple disease-modifying therapies (DMT) have been approved for the treatment of relapsing-remitting form of MS (RRMS). There are two approaches to treating MS. One approach is the continuous application of therapy (so-called maintenance therapy), which can then be optimized depending on the course of the disease. Maintenance therapy includes interferon beta, glatiramer acetate, teriflunomide, dimethyl fumarate, natalizumab, S1P receptor modulators (fingolimod), and B-cell therapies like ocrelizumab and ofatumumab. Another recent approach in the treatment of MS is immunoreconstitutional. Immunoreconstitutional therapy includes alemtuzumab and cladribine. It can be generally said that, with the exception of beta-interferon and glatiramer acetate, all other DMTs are associated with varying degrees of the risk of infection. It is this increased risk of infections in MS with various DMTs that has become very relevant due to the COVID-19 pandemic. It should be emphasized that people with MS do not have an increased risk of getting COVID-19 and that risk factors for developing severe COVID-19 disease is similar like in general population. There is also no scientific evidence that the DMTs we use to treat MS increase the possibility of infection or the course of COVID-19 infection, with the possible exception of corticosteroids and CD-20 depleting therapy. Another aspect with the same importance is response to vaccination, which is attenuated for many DMTs we use in treatment of MS. It is clear that any decision to initiate DMTs during the COVID-19 pandemic will need to be carefully made and will depend on the state of the COVID-19 pandemic, not only in the specific country but also in the specific area where the person lives and receives therapy. In doing so, care should be taken to take a proactive approach to MS treatment, focus on the person with MS at all stages of the disease in order to minimize the effects of the disease and maximize quality of life.

PRESIDENTIAL SYMPOSIUM “Current trends in neuroscience”

Dissecting pathogenic and protective mechanisms for Alzheimer's disease using human cerebral organoid models

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The use of induced pluripotent stem cells (iPSCs) for the generation of 3D cell culture models, and in particular cerebral organoids, is opening new possibilities for advances in the research into neurodegenerative diseases. For the first time, information can be gathered in a patient-specific manner on the biology of neurons before the onset of symptoms. So far, such information could only be inferred from brain imaging and CSF biochemical studies. While on one hand the mixed brain organoids lack brain cell types other than neurons (microglia, endothelial cells, podocytes, blood-derived macrophages), they have some advantages to studying biomarkers compared to CSF sampling. CSF measurements present the final product of a very complicated interplay of many factors that are difficult to dissect mechanistically. Mixed brain organoids allow for the detection of biological parameters that are intrinsic to the biology of neurons and neuronal networks in the first instance, free from the effects of most other cell types. Introduction of specific other cell types into the model can further dissect their cell-type specific roles. New compounds could be screened for their effects in a hypothesis-free manner. It is also easier to recruit patients/donors of primary cells (specially from healthy individuals), than sampling CSF.

We have established a Down syndrome (DS) cerebral organoid system that secretes amyloidogenic Aβ peptides in the picomolar range, and generates in vitro the triad of Alzheimer’s disease (AD)-like pathologies: amyloid plaque-like deposits, progressive spread of hyperphosphorylated, pathologically conformed and fibrillar Tau, and progressive neuronal loss. All of these 3 pathologies are pharmacologically preventable by the chemical inhibition of production of Aβ. In addition, toxic soluble Aβ aggregates and Tau aggregates can be measured and monitored in conditioned media from organoid cultures. Using this system, we have confirmed a dose-sensitive protective role for the chromosome 21 gene BACE2 (Alic et al., Mol Psych, 2020).

Reference: Ivan Alić, Pollyanna Goh, Aoife Murray, Erik Portelius, Eleni Gkanatsiou, Gillian Gough et al. Patient-specific Alzheimer-like pathology in trisomy 21 cerebral organoids reveals BACE2 as a gene-dose-sensitive AD-suppressor in human brain”, Molecular Pshychiatry, 2020: 10.1038/s41380-020-0806-5. Online ahead of print. PMID: 32647257

Synaptic plasticity in postnatal and adult hippocampal neurogenesis

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Evidence is increasing that neurogenesis of hippocampal granule cells is continuous throughout live in many mammals and particular in humans. Young granule cells are more plastic and therefore seen as an important source of hippocampal learning and memory formation. We show in adult rats under *in vivo* conditions, that newborn granule cells exhibit a gradual increase of synaptic plasticity, namely homosynaptic plasticity (LTP) within 4 weeks and heterosynaptic plasticity (LTD) within 5 weeks of cell age. Both LTP and LTD are observed in single retrovirally labelled new granule cells. In addition, 4-5 weeks old granule cells show forms of dendritic plasticity that is not present in mature granule cells. In organotypic hippocampal slice cultures we perform live imaging of postnatally born granule cells. Again, we find a higher dendritic plasticity and signs of gradual integration of newly born granule cells.

The interplay of mitochondrial and lysosomal dysfunction in Parkinson's Disease

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There is an urgent need to identify effective neuroprotective therapies for synucleinopathies such as Parkinson's disease (PD) and Diffuse Lewy Body Dementia (DLB). Recent emergence of genetic forms of PD has facilitated identification of potential targets for therapeutic development. As a general strategy, we are studying rare genetic diseases, in particular those with mutations in genes that play a role in these common pathogenic pathways (e.g. PINK1, Parkin, ATP13A2, GBA1, DJ-1) with a goal of identifying specific targets for therapeutic development in neurodegeneration. We found that the convergence of these pathogenic phenotypes in various forms of PD was mediated by dopamine oxidation that was detected in human neurons but not in mouse dopaminergic neurons.

Since dopamine oxidation leads to formation of neuromelanin, these findings also explained why neuromelanin is not normally found in mouse midbrain dopaminergic neurons. Moreover, these findings highlighted the importance of studying mechanisms of PD in patient-derived neurons and at least in part explained why animal models of genetic forms of PD do not exhibit degeneration of dopaminergic neurons that is observed in PD patients. Based on these findings, we developed targeted therapeutic approaches in human neurons that partially ameliorated pathogenic phenotypes in dopaminergic neurons from patients with multiple genetic and sporadic forms of PD. We recently identified the formation of direct mitochondria-lysosome membrane contacts that mark sites for lysosomal regulation of mitochondrial networks, while conversely, mitochondrial contacts regulate lysosomal dynamics providing a new angle to studies of these organelles in neurodegenerative diseases including PD.

Kinematic study of *in utero* fetal movements

Lana Vasung

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Brain-body interactions in adults are primarily achieved *via* continuous sensory-motor integration, with sensory representations serving a framework in which the motor system plans, coordinates, and executes motor programs resulting in movement. In contrast, little is known about sensory-motor integration in fetuses.

Fetal behavior, defined as any observable action or reaction, reflects the functioning and maturity of the central nervous system. Simple fetal movements start around seven gestational weeks (GW), i.e., when dorsal root fibers carrying sensory information from muscles and joints develop and reach the brain. Around 10GW, complex, highly variable spontaneous fetal movements (characterized by numerous combinations of flexions-extensions, abductions-adductions, and rotations) emerge. However, the descending pathways of the brain, which control spinal motor neurons and are responsible for the voluntary control of the musculature of the body, show protracted development. They reach the lower thoracic chord by 19 GW and the lumbosacral cord by 29 GW.

We used dynamic *in-utero* fetal MRI (N=52, 24-40 GW old) to characterize the fetal motion and conduct a kinematic analysis of fetal limb movement. We hypothesized that maternal, placental and fetal physiology and anatomy play a significant role in the early patterning of fetal motor behavior. Our results provide evidence that maternal and placental physiology (hyperoxia), possibly modified by anatomy (e.g., maternal, fetal or placental position during the scan), plays a significant role in the early patterning of fetal motor behavior and that the pattern of movements is different between upper and lower extremities before 30 GW.

THEMATIC SYMPOSIA

I. SLEEP AND SLEEP RESEARCH DURING THE COVID-19 PANDEMIC

Sleep and sleep research update in the COVID-19 pandemic

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The COVID-19 pandemic greatly influenced sleep medicine practice, as well as sleep research worldwide. But also, it had great impact on sleep in general population due to long-term home confinement and measures of social distancing and lockdown. Finally, many studies confirmed negative effect of COVID-19 disease on patients' sleep. In different studies the prevalence of sleep disturbances ranging from 33.3% to 84.7% in hospitalized COVID-19 patients.

Sleep disturbance may be associated with the adverse health consequences of COVID-19 patients and it continued to affect 29.5–40% of COVID-19 survivors during the early post-discharge period. Sleep disturbance, mental illnesses, and physiologic illnesses form a vicious cycle to worsen the prognosis in COVID-19 patients.

Sleep medicine practice was greatly affected due to closing of vast majority of sleep centers or sleep labs worldwide and majority of patients were left with insufficient health care. Many attempts to overcome the crisis using the telemedicine approach had only modest success and it is yet to be seen in the future what are the full consequences of this pandemic on sleep disturbed patients who heavily depend on the sleep medicine practice.

Sleep research during the pandemic was also greatly affected by the COVID-19, which became the focus of majority of studies in different areas of sleep research. One of the topics deserved special attention due to the fact that the SARS-CoV-2 virus infection is primarily causing the respiratory syndrome and the troubled breathing is typical clinical presentation of the COVID-19 disease, but many new evidences provide additional explanation that respiratory symptoms could be in part due to SARS-CoV-2 invading the respiratory centers of the brain. As the brainstem has a relatively high expression of ACE2 receptor compared with other brain regions, SARS-CoV-2 may exhibit tropism therein. The brainstem is also highly prone to damage from pathological immune or vascular activation, which has also been observed in autopsy of COVID-19 cases, as shown recently in areas like the brainstem that control the normal breathing process with nuclei like the pre-Bötzinger complex (pre-BÖTC), which may explain why some of the patients with COVID-19, who have been reported to have recovered from pneumonia, could not be weaned from invasive mechanical ventilation and the occurrences of acute respiratory arrests seen in COVID-19.

Finally, many new studies showed strong impact of disturbed sleep in so-called long-COVID and post-COVID patients. Insufficient sleep in those patients contributes to "brain fog" and the recent studies showed that improved sleep might significantly contribute to improvement of those symptoms.

Back to the basics: learning how redox modulation regulates sleep and addiction using animal model

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Proteins connected to redox balance regulation, either by producing or removing reactive oxygen species (ROS), have a role in the healthy cell, but many diseases are linked to disturbed redox balance, such as addiction or COVID-19 disease. Recent studies show that redox signaling is involved in the process of neuronal plasticity and that balance of redox intermediaries regulate sleep and wake transition in *Drosophila melanogaster*

One form of drug-induced neuroplasticity is locomotor sensitization (LS) that develops after repeated exposures to psychostimulans. To identify genes involved in the redox regulation that play a role in the development of LS we performed genetic screen using transgenic flies in which we knocked out genes using gene specific RNAi constructs and further characterize those mutants for: locomotor activity, vertical climbing, longevity and sleep. We identified genes that are required for the development of LS, those that significantly changed the amount of sleep, and some that affected both phenotypes. Panneuronal expression of RNAi always led to change in the sleep amount, but only some genes led to LS phenotype suggesting importance of redox regulation in sleep in most, if not all, brain areas.

Our results show that *Drosophila* is a versatile model organism for unbiased identification of genes involved in the regulation of cell functioning and in cellular changes that happen in disease. Role that redox regulation plays in different diseases suggests that simple dietary interventions could be devised to prevent or help disease symptoms.

Effects of lockdown during the COVID-19 pandemic on sleep and mental health

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The uncertain, ever-changing and an ongoing nature of the COVID 19 pandemic means that it may take some time before we can fully appreciate the negative effects of the pandemic and lockdown on our sleep and mental health. It is increasingly recognised that in the aftermath of a pandemic, several persistent sleep, neuropsychiatric and physical sequelae may continue long after the pandemic is over. To date, a body of evidence also highlights a significant disparity in sleep and mental health difficulties in specific vulnerable groups, with differing temporal profiles and sleep issues that are reported.

In this perspective, it is argued for a possible mechanistic impact of the COVID-19 pandemic, with its imposed restrictions and social isolation on sleep quality. I similarly discuss some of the potential international similarities and differences behind the reported idiosyncratic biological vulnerabilities that may have contributed to the genesis of sleep issues. Lastly, I propose some possible implementations and innovations that may be needed in restructuring sleep disorders services in order to benefit recovering COVID-19 patients.

Sleep and lifestyle habits during the COVID-19 pandemics in Croatia

Ivana Pavlinac Dodig

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Long-term home confinement and measures of social distancing and lockdown have influenced daytime routines. To evaluate effects of the COVID-19 lockdown on sleep habits, we performed a study on 1173 participants of general population in Croatia which revealed a strong negative impact of home confinement on sleep. We found that sleep latency prolonged from 10 (5-20) to 15 (10-30) minutes during COVID-19 lockdown ($P<0.001$). Moreover, there was a significant shift of ~38 min in bedtime and of ~58 min in waketime during lockdown ($P<0.001$).

To evaluate the influence of COVID-19 lockdown on lifestyle habits, we investigated a sample of 3027 participants of general population in Croatia. A total of 30.7% subjects reported to gain weight during the lockdown, with female gender and higher BMI being associated with an increased likelihood of gaining weight. On the contrary, reports of exercise before lockdown decreased the likelihood of gaining weight during COVID-19 lockdown.

Impact of COVID-19 lockdown was investigated in 652 medical and 511 non-medical students. We found a significant shift in bedtime and waketime in both groups, indicated by later bedtimes and wake-times ($P<0.001$). Both groups more frequently complained of insomnia, difficulties falling asleep and nighttime awakenings ($P<0.001$), while only medical students reported a decrease in tiredness during the lockdown ($P<0.001$). One might conclude that co-appearance of prolonged and delayed sleep time along with decreased tiredness among medical students during the lockdown implies their significant workload during pre-lockdown period.

Lastly, since OSA patients had an increased risk for severe COVID-19 forms and outcomes, we investigated lockdown-related changes in CPAP adherence among 101 severe OSA patients. We demonstrated that the average lockdown-related CPAP adherence has improved. However, despite the wide recognition of male gender and advanced age as risk factors for adverse COVID-19 outcomes, CPAP adherence improvements were more pronounced in women and younger respondents.

II. BRAIN LIPIDOMICS

Lipidomic analysis of demyelination associated with complex ganglioside deficiency

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Under normal conditions, maintenance of myelin relays on interaction between a-series (GD1a) and b-series (GT1b) gangliosides with myelin associated glycoprotein (MAG). Lack of b-series, like in *St8Sia1* $^{-/-}$ genotype, is not associated with myelin defects due to dose compensation with a-series. Lack of both, a- and b-series (*B4Galt1* $^{-/-}$ genotype), leads to late onset demyelination, despite of dose compensation with GM3 and 0-series gangliosides. Upon interaction with MAG, gangliosides recruit p75 neurotrophin receptor (p75NtR) and trigger signalling through RhoA pathway leading to two possible outcomes – growth cone collapse or cytoskeleton phosphorylation and neurofilament packing associated with thickening of axons. Disruption of this signalling leads to Wallerian degeneration and unwinding of oligodendrocyte extensions. In order to investigate which metabolic processes form the basis of this process, we used metabolomics and lipidomics analysis by imaging mass spectrometry. In the case of *B4Galt1* $^{-/-}$ mice we identified overexpression of metabolites related to ferroptosis. The opposite was found in *St8Sia1* $^{-/-}$ mice in which, in addition to ferroptosis metabolites, the synthesis of porphyrins, ubiquinones, arginine, proline and phenylalanine was reduced. Also, the plasma membrane of these mice contained less cholesterol and phospholipids, but there was a higher content of actin filaments in the cytoplasm. It is to be expected that changes in the oxido-reductive balance in both animal models ultimately lead to neurological deficits. Ferroptosis is a probable cause of oligodendrocyte death in the *B4Galt1* $^{-/-}$ phenotype, while in the *St8Sia1* $^{-/-}$ the most affected cellular populations have yet to be determined in more detail.

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Disruption of brain sterol biosynthesis by commonly used prescription medications

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The brain is the most cholesterol rich organ of the body. Brain development and cholesterol accrual are closely connected processes. During intrauterine development the fetal brain synthesizes its own cholesterol, independent of fetal systemic cholesterol synthesis and maternal supply. Although cholesterol cannot cross the blood-brain barrier, maternal intake of medications that cross the placental and fetal blood-brain barriers can alter developmental sterol brain biosynthesis, with highly detrimental effects. Pharmaceutical inhibition of sterol synthesis closely mimics genetic disruptions that lead to developmental disorders, including (but not limited to) Smith-Lemli-Opitz syndrome (SLOS). The use of medications in pregnancy is ubiquitous. Our high-throughput screening (HTS) of approximately 5,000 approved pharmaceuticals in Neuro2a cell lines identified that approximately 5% of the tested compounds altered sterol synthesis *in vitro*. *In vivo* mouse experiments and patient biobank assessments confirmed our *in vitro* findings that aripiprazole, cariprazine, haloperidol, trazodone and amiodarone are strong inhibitors of post-lanosterol biosynthesis. Many of the currently used medications have not been evaluated for safety during pregnancy, and the long-term consequences of fetal exposure remain unknown. Our studies will elucidate the effects of commonly used prescription medications on sterol synthesis and identify drugs and drug combinations that should be used with extreme caution during pregnancy.

Interaction of genetics, pregnancy and medications on the developing brain

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Cholesterol is essential for normal brain function and development. Genetic disruptions of sterol biosynthesis result in intellectual and developmental disabilities. Developing neurons synthesize their own cholesterol, and disruption of this process can occur by both genetic and chemical mechanisms. Many commonly prescribed medications interfere with sterol biosynthesis, including haloperidol, aripiprazole, cariprazine, fluoxetine, trazodone and amiodarone. When used during pregnancy, these compounds might have detrimental effects on the–developing brain of the offspring. In particular, inhibition of dehydrocholesterol-reductase 7 (DHCR7), the last enzyme in the biosynthesis pathway, results in accumulation of the immediate cholesterol precursor, 7-dehydrocholesterol (7-DHC). 7-DHC is highly unstable, giving rise to toxic oxysterols; this is particularly pronounced in a mouse model when both the mother and the offspring carry the *Dhcr7*$^{+/-}$ genotype. Studies of human dermal fibroblasts from individuals who carry *DCHR7*$^{+/-}$ single allele mutations suggest that the same *gene*medication* interaction also occurs in humans. The public health relevance of these findings is high, as DHCR7-inhibitors can be considered teratogens, and are commonly used by pregnant women. In addition, sterol biosynthesis inhibiting medications should be used with caution in individuals with mutations in sterol biosynthesis genes, which are present in 1-3% of the human population. In an age of precision medicine, further research in this area could open opportunities to improve patient and fetal/infant safety by tailoring medication prescriptions according to patient genotype and life stage.

Gangliosides framework for optimal function of plasma membrane ion transporters

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Lipid-protein interactions determine the proper positioning and function of plasma membrane proteins. Plasma membrane is not a simple homogeneous cellular envelope, but a highly dynamic structure resembling a patchwork of lipid rafts microdomains with distinct composition and physicochemical properties. These nanometer-scale submembrane compartments are enriched with (glyco)sphingolipids, cholesterol, and a select subset of transmembrane proteins. Hence, lipid-protein interdependence and functional interplay is markedly highlighted in these domains. Gangliosides, the most complex glycosphingolipids, have many documented effects including modulation of ion homeostasis. Since the neuronal membrane shows the highest concentration and compositional diversity of gangliosides, their effect on plasma membrane ion transporters involved in membrane potential generation and maintenance is of special interest. Gangliosides are shown to have effects on Na^+, K^+-ATPase (NKA), as well as plasma membrane calcium-ATPase (PMCA). Depending on the number of sialic acids present in the ganglioside structure, they can have stimulatory or inhibitory effect on PMCA. Therefore, the complexity of ganglioside structures can fine-tune PMCA activity and affect the Ca^{2+} ions extrusion from the cell. Our group has shown that specific gangliosides are necessary for providing the appropriate structural framework for the positioning and consequently normal function of PMCA, complexed with its essential auxiliary subunit, glycoprotein neuroplastin. In addition, the dual role of gangliosides in the supramolecular architecture of submembrane domains is documented for NKA as well. As we have shown, gangliosides GD1a, GD1b and GT1b having notably richer topology may serve as modulators of NKA activity, while GM1 may have a structurally supporting role for NKA positioning and assembling within the lipid rafts or bulk membrane microdomain. Disturbances in the lipid environment of the membrane are well recognized as potential driving factors in the pathogenesis of several human disorders. However, the mechanism leading from changed ganglioside composition of the membrane to the clinical presentation of these diseases remains an enigma. Hopefully, the work elucidating the exact effect of gangliosides on plasma membrane ion transporters in the brain will facilitate understanding of ethiopathology of these complex disorders, as well as contribute to fundamental knowledge of ion homeostasis regulation in the brain.

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III. ALZHEIMER'S DISEASE

Prooxidative effect of flavonols in copper-induced oxidative stress

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Neurodegenerative diseases are characterized by the progressive loss of specific populations of neurons. Oxidative stress (OS), induced by increased accumulation of reactive oxygen species (ROS), is one of the pivotal mechanisms underlying the common pathophysiology of these diseases. OS may be induced by disturbed homeostasis of transient metal ions that initiate production of ROS. Based on their ability to act as ROS scavengers and metal chelators, natural polyphenolic compounds are appreciated as potential neuroprotective agents. We investigated effects of flavonols quercetin and myricetin against copper-induced neuronal death in human neuroblastoma cell line SH-SY5Y and P19 neurons. In both cellular systems, flavonols were capable to exacerbate toxic effects of copper and promote death. In P19 neurons, effects of quercetin were determined by the severity of neuronal injury. In moderate OS, quercetin exerted neuroprotective effect by attenuating ROS production, expression of PUMA and downstream apoptotic events (caspase-3 activation, nuclear condensation). During the severe oxidative insult, quercetin demonstrated prooxidative effect detrimental for neuronal survival. In neuroblastoma cells, only prooxidative action of myricetin was observed ending in caspase-independent programmed cell death and necrosis. Treatment with myricetin increased changes in chromatin condensation and damage of the plasma membrane. At the protein level, myricetin upregulated expression of PARP-1 and decreased expression of antiapoptotic protein Bcl-2. Atomic force microscopy revealed myricetin-induced changes of morphological (cell surface) and nanomechanical properties (roughness, elasticity). Hence, further studies should be carried out to better characterize possible side effects of prolonged supplementation with flavonols, particularly in conditions accompanied with metal-induced OS.

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The role of proton magnetic resonance spectroscopy in diagnostics and investigating the disease course and treatment response in Alzheimer's disease/Uloga protonske spektroskopije magnetskom rezonancijom u dijagnozi Alzheimerove bolesti, praćenju tijeka bolesti i praćenju terapijskog odgovora

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Protonska spektroskopija magnetskom rezonancijom (1H-MRS) je ne-ionizirajuća, neinvazivna, *in vivo* metoda koja se sve češće koristi u praćenju promjena neurometabolita u mozgu u nizu psihijatrijskih i neuroloških poremećaja, uključujući Alzheimerovu bolest (AB). 1H-MRS bi mogla omogućiti objektivne, *neuroimaging* parametre koji bi se mogli koristiti u dijagnozi Alzheimerove bolesti, u praćenju tijeka bolesti, te u praćenju terapijskog odgovora na antidementive.

Dijagnoza AB se najčešće postavlja u stadiju uznapredovalih neuropatoloških promjena. Promjene na 1H-MRS su već vidljive kod ranih kognitivih tegoba, npr. u blagom kognitivnom poremećaju. Rana dijagnoza AB bi tako omogućila ranije intervencije s većom vjerojatnosti terapijskog učinka.

U istraživanjima AB najčešće se nalaze promjene N-acetil aspartata (NAA), mioinozitola (MI) i kolina (Cho). NAA se smatra markerom funkcije i gustoće neurona. MI predstavlja marker aktivnosti glije. Signal kolina na MRS se sastoji od slobodnog kolina te predstavlja membranski marker, odnosno marker membranskog *turnovera.*

U AB nalazimo smanjenje NAA u posteriornom cingulumu i hipokampusu, smanjenje NAA/Cr u posteriornom cingulumu, povećanje MI/Cr u posteriornom cingulumu i parijetalnoj sivoj tvari, povećanje MI i MI/NAA u posteriornom cingulumu, te smanjenje Cho i Cho/Cr u posteriornom cingulumu.

Neke studije pokazuju korelaciju promjena razine metabolita ili omjera metabolita i kognitivnog testiranja u praćenju AB. Dio studija nalazi promjene u koncentraciji neurometabolita pri terapiji antidementivima koje nisu nužno uvijek u korelaciji s terapijskim učinkom.

Nastavak istraživanja na tehnološki naprednijim MR aparatima te analizom apsolutnih koncentracija metabolita umjesto omjera metabolita pružaju mogućnost da 1H-MRS u kombinaciji s drugim metodama doprinese napretku u razjašnjavanju etiologije te u praćenju progresije AB.

The relationship of *APOE* genotype with essential metals in Alzheimer's disease

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There are many existing hypotheses that are trying to explain the development of Alzheimer's disease (AD). One of these hypotheses is hypothesis of altered metal homeostasis in the AD brain. This hypothesis mainly refers to essential metals that are normally present in the body and are necessary for normal functioning of many proteins. The gene for apolipoprotein E (ApoE), a protein involved in transport of cholesterol to neurons, is the major genetic risk factor for late-onset, sporadic AD. The scope of this study was to investigate possible association between ApoE and essential metals. We measured cerebrospinal fluid (CSF) and plasma levels of iron, copper, zinc, magnesium, sodium, cobalt, calcium, manganese, molybdenum, boron and chromium, and CSF ferritin levels among AD, mild cognitive impairment (MCI) patients and healthy controls (HC) with different *APOE* genotype. Plasma levels of copper, sodium and magnesium were increased in patients carrying ε4 allele. The increase in plasma levels of sodium, cobalt and calcium was observed in patients carrying ε4εx genotype. Plasma boron levels were decreased in carriers of ε4 allele and ε4ε4 genotype. Additionally, CSF zinc levels and sodium plasma levels were increased in AD patients in comparison HC. In conclusion, these results indicate a strong association between *APOE* genotype and plasma levels of sodium, copper, zinc, calcium, magnesium and cobalt, as well as the metalloid boron in AD and MCI patients.

Different effects of acute central inhibition of the glucagon-like peptide 1 and gastrointestinal inhibitory peptide receptor in a rat model of sporadic Alzheimer's disease

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Among the more promising treatments proposed for Alzheimer's disease (AD) are those affecting incretins. The goal was to explore the central effects and peripheral hormone levels of the acute central GLP-1 receptor (GLP-1R) and GIP-receptor (GIP-R) inhibition in a rat model of AD (streptozotocin-intracerebroventriculary treated rats,STZ-icv). One month after STZ-icv (3 mg/kg) treatment male Wistar rats were icv treated with vehicle or GLP-1R antagonist (Exendin (9-39) amide; Ex9) or GIP-R antagonist (Pro-3 GIP) and sacrificed 30 minutes afterward. Protein expression of c-fos (marker of neuronal activation), AMP-activated protein kinase, total and phosphorylated form (tAMPK; pAMPK) in hippocampus (HPC) was measured by Western blot. Insulin, GLP-1 and GIP concentration was measured by ELISA and glucose by GOD-PAP standardized test in plasma. Data were analyzed by Kruskal-Wallis one-way ANOVA and Mann-Whitney U test ($p<0.05$). Only Ex9 treatment increased the expression of c-fos ($p<0.05$) while both inhibitors decreased the ratio of p/t AMPK in HPC of STZ-icv treated rats, The glucose and insulin plasma concentration remained unchanged after Ex9 but insulin was two-time higher after Pro-3 GIP . The concentration of active GLP-1 form was found increased in STZ-icv treated rats compared to control while Ex9 decreased its concentration. The Pro-3 GIP increase the GIP concentration in both treated groups. Central GLP-1R and GIP-R inhibition affects both brain and periphery demonstrated by distracted hippocampal energy homeostasis accompanied by increased GIP plasma level. The results emphasise the importance of central incretin system in the AD pathophysiology and development of new potential incretin-based drugs.

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The role of arterial spin labeling magnetic resonance imaging technique in the diagnosis of alzheimer's disease – our initial findings

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ASL (arterial spin labeling) is a non-invasive MR imaging technique to measure cerebral blood flow (CBF). Well-established methods measure CBF by dynamic imaging of the passage of a contrast agent. By contrast, ASL generates an image by magnetically “labeling” water molecules as an endogenous tracer as they travel to organ of interest. CBF values obtained with ASL correlate with golden standard PET. For Alzheimer's disease diagnosis two regions that should be thoroughly observed are posterior cingulate cortex and precuneus. 5 patients with Alzheimer's disease were scanned on MRI at Clinical Hospital "Sveti Duh" using 3.0-T Siemens Magnetom Vida scanner. Inclusion criteria were confirmed diagnosis of Alzheimer's disease (based on NIA-AA clinical criteria and Hachinski ischemic score) and age of subjects 50 and above. The degree of cognitive impairment was assessed with MMSE (<23) and MoCA (<26). Non-inclusion criteria were internal carotid artery stenosis > 70%, cardiac decompensation (NYHA IV), ischemic stroke in posterior cingulate cortex and precuneus, aphasia, contraindications for MRI scan, reversible causes of dementia, mixed dementia and other types of dementia. Exclusion criteria were phobia, inability of subjects to lay down calmly and other brain diseases that affect cognitive function. Obtained perfusion maps showed that all 5 patients with Alzheimer's disease have decreased perfusion in posterior cingulate cortex and 4 out of 5 patients with Alzheimer's disease have decreased perfusion in precuneus. Preliminary results showed that ASL imaging technique can have important role in the diagnosis of Alzheimer's disease based on specific pattern of perfusion in Alzheimer's disease.

Dehydroepiandrosterone and brain-derived neurotrophic factor: potential therapeutic targets in Alzheimer's disease/ DHEA(S) i BDNF - potencijalni terapijski ciljevi u Alzheimerovoj bolesti

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Neurosteroidi dehidroepiandrosteron (DHEA) i njegov sulfat (DHEAS), zajedno nazivani DHEA(S), igraju važnu ulogu u neuroprotekciji, rastu neurita, neurogenezi, apoptozi, metabolizmu kateholamina i imunološkom odgovoru, te pokazuju antiupalno, antioksidativno i antiglukokortikoidno djelovanje. Neurotrofin moždani neurotrofni čimbenik (BDNF) također je uključen u važne funkcije mozga poput neuralne plastičnosti, učenja, pamćenja i ponašanja. Usprkos potencijalu DHEA(S) i BDNF u prevenciji i liječenju Alzheimerove bolesti (AB), te studijama njihovih učinaka na staničnim i animalnim modelima, te bolesnicima, uloga BDNF-a i DHEA(S) u AB nije jasna, te su neophodna daljnja istraživanja. U našim istraživanjima pokušali smo razjasniti stanične i mol. mehanizme neuroprotektivnog djelovanja DHEA(S) i BDNF u primarnoj kulturi neurona tretiranih Aβ oligomerima, kao in vitro modelu AB. Dobiveni rezultati koji upućuju na antiapoptotsko djelovanje DHEA(S) i BDNF potvrditi će se i nadopuniti u in vivo i ex vivo istraživanjima u kojima ćemo pratiti ponašanje i kognitivne funkcije i njihovu korelaciju s histopatološkim, neurokemijskim i strukturnim promjenama u mozgu transgeničnog mišjeg modela 3XTg-AD. Na animalnom modelu će se odrediti metabolomski profil kao potencijalni biomarker za dijagnostiku AB, specifičnih simptoma i odgovora na terapiju s DHEA(S). U bolesnika s AB istražiti će se genetički i epigenetički čimbenici uključeni u ekspresiju i regulaciju BDNF i DHEA(S). Rezultati istraživanja trebali bi proširiti znanje o neurobiološkoj podlozi kompleksne stanične i molekularne patofiziologije AB, doprinijeti boljem razumijevanju terapijskog potencijala DHEA(S) i BDNF, kao i razvoju lako dostupnih biokemijskih, metabolomskih i (epi)genetičkih biomarkera, te novih pristupa u prevenciji, praćenju i liječenju AB.

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IV. PRECLINICAL MODELS OF NEUROLOGICAL DISEASES

Application of stem cells in brain diseases: focus on mitochondria and cell death

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Faced with a burden of brain diseases, modern medicine searches for new therapeutic strategies. Here we present approach arising from technology of stem cells. In the last twenty years it has been shown that molecules which stem cells secrete act on various elements of molecular pathophysiology. Among many mechanisms reported, here we focus on those linked to cell death and survival of mitochondria. Based on *in vitro* model of hypoxia, we showed that lack of oxygen influences integrity of mitochondria, often leading to mitophagy and triggering cell death. Stem cells and especially their secretions in the form of exosomes very powerfully revert some of these negative events. Moreover, it seems that among many types of cell death linked to hypoxic damage, like apoptosis, necroptosis and ferroptosis, some are more efficiently mitigated by stem cells than others. Since possible applications of this approach are numerous, it is expected that elements of stem cells secretome will continue to shape a completely new field of pharmacology focused on treatment of brain diseases.

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Opossum *Monodelphis domestica* in research of neuroregeneration and neurodegeneration

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One of the major challenges of modern neurobiology concerns the inability of the adult mammalian central nervous system (CNS) to regenerate and repair itself after injury. It is still unclear why the ability of neuroregeneration is lost during evolution and development and why it becomes very limited in adult mammals. A preferred model to study and reveal the cellular and molecular basis of this loss is neonatal opossum *Monodelphis domestica*. Opossums are marsupials that are born very immature with unique possibility to successfully regenerate spinal cord after injury in the first two weeks of their life. We have developed new *in vitro* platforms from CNS of immature opossums and revealed that activating transcription factor 3 (ATF3), and possibly other members of regeneration-associated genes (RAG) and ATF/ cAMP - response element binding (CREB) family of transcription factors, have the important role both during cortical postnatal development and in response after injury. Next, using comparative proteomic approach we identified the proteins unique and differentially distributed in opossum spinal tissue with different regenerative capacities, with emphasis on proteins related to neurodegenerative diseases.

Rat model of chronic stress – connection to neurodegeneration

Marta Balog

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Chronic stress produces long-term metabolic changes through the superfamily of nuclear receptors, resulting in various pathologies. Glucocorticoids are easily crossing the blood-brain barrier, influencing the central nervous system by binding to their receptors, highly concentrated in hypothalamus, cortex and amygdala – the same regions severely disrupted in Alzheimer's disease (AD). Animal models of chronic stress have been widely used in many different pathologies, including neurodegeneration. We have learned from such models that stressed animals elicit altered behavior, gene expression, functional connectivity and hippocampal volume similar to those in patients suffering from neurodegeneration. Particularly, memory decline, as a specific behavioral stress response in Sprague Dawley rats was recorded by passive avoidance test, with premenopausal females manifested stronger cognitive decline than males of the same age. Neuroinflammation, amyloidogenesis, and disrupted neuroplasticity were also more specific to stressed females while increase of tau expression was observed in stressed male rats. Stress caused cellular membrane disorganization in hippocampus of stressed female rats, a phenomenon affecting the trafficking of molecules associated with synaptic plasticity, such as APP, AMPA-R, and neuroplastin. Cognitive dysfunction upon stress in reproductively senescent females might be underlied by expressional changes in the lipid environment of APP, neuroplastin, AMPA-R, glial cell number, as well as structural changes of the membrane lipids while in males it might be driven by different mechanisms. Both environmental and genetic factors have an important role in the onset and risk for neurodegenerative diseases with animal models offering a great tool for revealing the complex mechanisms and developing potential therapies.

Retinal ischemia in rodent stroke models

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Approximately 65% of ischemic stroke incidents are accompanied by visual impairment. In humans the ophthalmic artery (OA) originates from the internal carotid artery (ICA) proximal to the origin of the middle cerebral artery (MCA), thus occlusion of the MCA should result in retinal damage in animal stroke models, which is not always the case. The aim of our study was to clarify which modifications of MCA occlusion induce retinal ischemia by longitudinal *in vivo* magnetic resonance assessment of cerebral and retinal vascular perfusion and the resulting cerebral and retinal ischemic injury. To compare brain and retinal ischemic lesion development two MCAO approaches, modified Koizumi and Longa were applied. The animals were longitudinally scored for neurological deficit followed by magnetic resonance imaging. Most of the retinal responses to ischemia overlapped with those in the brain when the Koizumi method is performed. The short occlusion with prolonged hypoperfusion resulted in simultaneous brain and retinal ischemia. Prolonged hypoperfusion in sham animals was sufficient to induce transient retinal changes. The Longa method resulted only in brain ischemia which was more extensive and resulted in greater tissue loss compared to the Koizumi method.

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What can rats tell us about neuropathic pain?

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Neuropathic pain is a debilitating disease of the somatosensory system that has a huge socioeconomic impact. Its pathogenesis is incompletely understood and treatments are often inadequate. Exploration of the mechanisms producing neuropathic pain has been aided by the interventions targeted to dorsal root ganglion (DRG). The DRG is located between the dorsal root and the spinal nerve. It contains pseudounipolar neurons that convey sensory information from the periphery to the CNS. Numerous studies, including those from our laboratory have established that the injured DRG is the important site for pathophysiologic changes that lead to development of neuropathic pain. A strong impetus for developing a suitable model of DRG targeted therapy is the avoidance of systemic and CNS toxicity during neuropathic pain treatment. Although DRG neurons are critical for the onset of neuropathic pain, relevant clinical treatments for neuropathic pain that target this organ are scarce. The neurostimulation techniques i.e. application of electrical currents to the target area using implanted electrodes can be successfully used to manipulate neuronal function at the level of injured DRGs. Although these procedures are already used in clinical practice we still know very little about therapeutic mechanism and potential adverse effects. One of the potential therapeutic mechanisms is related to filtering properties of DRG T-junction which builds on our previous study in which we showed that neuronal injury may disable T-junction filtering and thereby increase the net conduction of afferent traffic.

Repairing the mouse brain after stroke

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Clinical relevance of the preclinical mouse model of the stroke is a crucial issue in developing new interventions leading to the needed therapies. Several steps were introduced in our approach to improve the clinical relevance when using mice to learn more about human stroke. The animals are considered as patients and they are followed after ischemic brain lesion for 28 days. The monitoring of the animals during this period is based on *in vivo* imaging of the living animals. Magnetic resonance imaging contributes primarily to the structural insight in the evolution of the ischemic lesion. Bioluminescence imaging reveals the molecular activity of the luciferase bearing transgenes reflecting the expression of the endogenous genes. The imaging is correlated to the functional outcomes represented as neurological scores. This approach allows to compare the extent of the brain repair after ischemic brain lesion in the mouse, and subsequently can be used to compare eventual candidate interventions for the human therapies.

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POSTER PRESENTATIONS

P1 Basic neuroscience

PP1

OPTOELECTRONIC INDUCTION OF FIELD EXCITATORY POSTSYNAPTIC POTENTIAL BY ORGANIC PHOTOCAPACITOR

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Non-optogenetic stimulation of neurons by light has the potential to become a novel, minimally invasive, wireless, and highly specific in terms of time and space method for electrophysiological manipulation. Organic electrolytic photocapacitor (PhotoCap) is a semiconductor device that creates an *in situ* electrical field when stimulated by light and is stable in physiological conditions. It was previously shown that PhotoCap can stimulate neurons in culture and *in vivo* extracellularly. We performed field excitatory postsynaptic potential (fEPSP) recording that was stimulated by PhotoCap on mouse hippocampal brain slices. The PhotoCap was placed in the CA3 region for Schaffer collateral stimulation and the recording electrode was placed in the pyramidal layer of CA1 region. Stimulation of the device was achieved by delivering pulses of red light through the microscope objective. After light stimulation of the PhotoCap, we recorded responses which are most likely fEPSPs. Duration of the responses was approximately 5 ms and the amplitude 0,3 mV. Our preliminary results indicate that PhotoCaps can be used as stimulation devices in acute brain slices. The nature of the PhotoCap, i.e. its stability in physiological conditions, low invasiveness, high specificity, and its many potential uses allows for advancements in the field of fundamental research and medical applications. The PhotoCap proves to be a new valuable tool in neurophysiological research, especially electrophysiology. New applications, such as stimulation of specific brain regions and neurons lower in the neuronal pathway *in vivo* and/or changing the PhotoCap response by changing the light pattern, are yet to be performed.

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PP2

ROSTRO-CAUDAL DIFFERENCES IN THE RATIO OF GABAERGIC NEURONS SUBTYPES THROUGH THE RAT NEOCORTEX

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GABAergic neurons (GABAn) represent 20% of cortical neurons and are a highly diverse group that can be classified using molecular markers: the parvalbumin-expressing neurons account for about 40% of total cortical GABAn population in rodents, around 30% are somatostatin-expressing and 25% are calretinin-expressing neurons. Most of calbindin-expressing GABAn are also somatostatin positive, but significant proportion of somatostatin neurons does not co-express calbindin. However, the level of overlap between the aforementioned interneuron subpopulations is still controversial since some studies have shown additional co-expression between above mentioned markers. In this study we performed systematic assessment of their number and laminar position in frontal, parietal and occipital cortical region to assess proportion of different subclasses and the level of overlap between calretinin neurons and the remaining three subpopulations. A comprehensive qualitative analysis of double-labeled immunofluorescent histological sections showed that there were no major rostro-caudal differences in the number and laminar distribution of calbindin, parvalbumin and somatostatin neurons, while calretinin neurons were more abundant in occipital region. No overlap between calretinin and other major interneuron populations was observed. Parvalbumin, somatostatin and calretinin neurons were evenly distributed within a cortical column, while calbindin neurons were more numerous in upper cortical layers. In conclusion, the laminar distribution of GABAergic interneurons does not differ substantially between rostral and caudal cortical regions, however, calretinin neurons are generally more abundant in caudal than in rostral regions. Data also pointed on the significant difference in proportion and distribution of GABAn subtypes between rodents and primate cerebral cortex.

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PP3

MALE WISTAR RATS RAPIDLY HABITUATE TO CAT ODOUR PREDATOR STRESS TEST FOR ANXIETY

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To investigate whether Wistar rats habituate to predator stress test based on cat odour, we assigned 12 Wistar male rats to the group exposed to cat bedding dirtied with cat excretions (cat odour) and to the group exposed to clear, unuse cat bedding (false cat odour). Animals were tested in a square arena virtually divided by video tracking software in an unsafe, smaller zone containing a beaker filled with cat odour and a safe, large area with a cup filled with false cat odour. Individual rats could freely explore the arena for 10 minutes in habituation (it was empty), test (scants were in it) and retest session (same as test session). During the habituation session, we didn't find differences between experimental groups in behavioural data collected. But, during the test session, the number of contacts with the beakers and the number of rearing's decreased in the cat odour group, while the number of stretch attendings increased in this group compared to the false cat odour group. Also, the number of entries and time spent in unsafe zone declined and the total distance travelled in the arena enlarged in rats exposed to cat odour compared to rats exposed to the false scent. Behavioural and locomotor differences described above vanish during the retest session. Stretch attendings number was an exception. In rats exposed to cat odour, stretches still increased. Here we demonstrate that predator stress test based on cat odour is functional in our hands and susceptible to one trial habituation.

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PP4

GRANULE CELLS EXHIBIT REDUCED SPINE DENSITY AND HOMEOSTATIC CHANGES OF DENDRITIC SPINE PARAMETERS IN THE DENTATE GYRUS OF MICE LACKING TUMOR NECROSIS FACTOR (TNF)

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Dendritic spines are small extensions emanating from the dendrites of neurons. They are hosting excitatory, glutamatergic synapses and the size of their head is tightly correlated with AMPA receptor density and, thus, synaptic strength. Since tumor necrosis factor (TNF), an inflammatory cytokine, influences synaptic transmission as well as Hebbian and homeostatic forms of synaptic plasticity in physiological concentrations, and since synaptic strength and spine geometry are linked, we hypothesized constitutive TNF-deficiency should cause structural changes at the level of dendritic spines. To address this question, we used fixed sections of adult age-matched male TNF-KO and wildtype littermate mice and injected dentate gyrus granule cells with the fluorescence dye Alexa 568 hydrazide. Sections were also immunolabeled for the actin-modulating protein Synaptopodin (SP), an essential component of the spine apparatus. SP has been linked to Hebbian and homeostatic plasticity and to increased spine stability. Dendritic segments located in the outer molecular layer were imaged using a confocal microscope and the size of individual dendritic spines was analyzed and their SP-content was determined. Spines were divided into three categories with regards to size, comparing small, medium, and large-sized spines between the genotypes. TNF-KO animals exhibited a reduction in the density of dendritic spines which was compensated by an increase in the size of large-sized spines containing large SP clusters. These changes mimic homeostatic adaptations seen after denervation of granule cells, suggesting a similar mechanism in TNF-KO mice. Using TNF-R deficient mice, the downstream signaling pathways involved in these adaptations are currently under investigation.

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PP5

TRANSCRIPTION FACTORS IN THE DEVELOPING HUMAN SUBTHALAMIC NUCLEUS

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The subthalamic nucleus (STN) is one of the major output nuclei of the basal ganglia circuits. With the advancement of neurosurgical techniques for treatment of movement disorders, it has become necessary to explore the STN on a cellular and molecular level. Although the physiology and connectivity of the STN are well-described in model animals, cytoarchitecture, developmental origin, and molecular markers are still greatly underexplored, especially in humans. Developmental origin and transcriptional codes for the specification and maturation of STN neurons are still being debated. Moreover, most developmental studies have been carried out on animal models, but the results cannot be simply extrapolated to human brain. To gain a better understanding of processes involved in the development of human STN, we analyzed the expression of several transcription factors in the fetal STN during midgestation (15-20 post-conceptional weeks, PCW). Human fetal tissue was formalin-fixed, processed for paraffin-embedding, and cut at 10 µm. The expression of transcription factors (i.e. Foxp2, Foxp1, Foxa1, Barhl1, Pax6…) was studied using standard immunohistochemical methods. To the best of our knowledge, this is the first description of these transcription factors in the developing human STN. Our preliminary data indicate these trancription factors are important determinants of neuronal identity in the developing human STN and further studies considering their spatio-temporal expression throughout the embryonic and fetal period are needed.

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PP6

A COMPARATIVE ANALYSIS REVEALS INTER-SPECIES PHENOTYPIC DIFFERENCES IN THE ADULT MAMMALIAN SUBTHALAMIC NUCLEUS

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The subthalamic nucleus (STN) is a diencephalic structure which is an important part of the basal ganglia circuitry. As such, it has gained clinical interest as a target for deep brain stimulation treatment of Parkinson's disease. Although it is a clinically relevant structure, little is known about its basic cytochemistry and phenotypical profile. When looking at the existing data, results from different species are often not overlapping and data from one species cannot be extrapolated to another. Because of this, we decided to do a systematic comparison between three species most commonly involved in STN research (human, mouse and rat) and compare the expression profiles of different transcription factors in the adult STN between the species. Our results show that there are many differences in the expression profiles of transcription factors between the three species, and that the STN is not strictly phenotypically conserved among them. Since transcription factors control many other downstream genes, differences in their expression suggest that there is a whole other spectrum of differences in the downstream genes, which raises a question of what is their functional significance, and why has it diverged in different mammals' STN. Another concern is the fact that there are many experiments conducted with animal models of STN-related research and caution is needed in translating the results to humans since human and rodent STN might not quite be the same.

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PP7

TRANSIENT ROLE OF THE SUBPLATE NEURONS IN DIFFERENTIAL INGROWTH OF PULVINOCORTICAL AND GENICULOCORTICAL AXONS INTO THE PROSPECTIVE STRIATE AND EXTRASTRIATE CORTEX OF THE HUMAN FETAL BRAIN

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The final development of cortical arealization and function establishment requires complex mechanisms, such as the interaction of axon guidance molecules and morphogenes in a strictly defined spatio-temporal frame. In the future visual (striate, extrastriate) cortex, cytoarchitectonic parcellation was extensively studied in the context of histogenetic and neurogenetic processes within the transient fetal zones. We analyzed 7 postmortem human fetal brain samples from 12 to 17 postconceptional weeks (PCW) using classical histological methods, immunohistochemical and immunofluorescent cellular and fibrillary stainings to study cellulo-fibrillar architecture and its developmental dynamics regarding the establishment of the prospective visual neocortex. Our study revealed a conspicuous cellular "corridor" monolayer present during the 13-15 PCW, positioned at the interface of the intermediate zone and deep subplate. It occupies a caudo-ventro-medial position regarding the three-dimensional axis of the developing occipital lobe, approaching but never reaching the incipient calcarine fissure. Immunohistochemistry and double-labeling immunofluorescence revealed that "corridor" cells are NeuN+, Tbr1+, Cplx3+, VLGUT1+, EphA6+, Sema-3A+, GFAP- with an underlying Fibronectin+, Synaptophysin+, SNAP25+ fibrillary zone, speaking in favor that they are subplate neurons expressing axon guidance molecules, positioned above the growing axons of the intermediate zone. Considering their position towards the incipient calcarine fissure and guidance molecule expression, they are likely to have a transient role in the navigation of pulvinocortical axons to the prospective extrastriate area and the repulsion of geniculocortical axons to reach their final destination - the prospective striate cortex. This study confirms the importance of the tangential continuum of subplate neurons, this time in the context of precise differential target-oriented growth of massive visual projections.

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PP8

VENTRO-DORSAL DIFFERENCES IN PROPORTIONS OF GABAERGIC INTERNEURON POPULATIONS IN THE HUMAN PREFRONTAL CORTEX

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GABAergic interneurons are a diverse group of cortical neurons commonly identified with the following molecular markers – calretinin, parvalbumin, calbindin and somatostatin. We performed quantitative and qualitative analysis of these interneuron populations in dorsal (Brodmann area 9) and ventral (Brodmann area 14r) regions of the human prefrontal cortex (PFC) on five adult specimens using double labelling immunofluorescence. The interneuron markers were combined with each other as well as with NeuN. The proportion of each interneuron population within the total neuron population in both Brodmann areas (BA) was compared using a paired t-test. The analysis revealed substantial differences in ratios of interneuron populations between supragranular layers but not between infragranular layers. Calretinin neurons were the most numerous interneuron population in supragranular layers and somatostatin neurons were the most numerous in infragranular layers. Furthermore, somatostatin and calbindin neurons had different laminar molecular profiles – in supragranular layers, the level of somatostatin and calbindin co-expression was high, whereas in infragranular layers the level of co-expression was extremely low. The proportion of calretinin neurons was significantly higher in BA14r (average: 10.24%) than in BA9 (average: 8.21%). The proportions of other interneuron populations did not differ significantly between the two cortical regions, though the proportion of somatostatin neurons was on average higher in BA14r than in BA9. In addition, the ratio between calretinin and parvalbumin neurons was significantly higher in BA14r (average: 1.62) than in BA9 (average: 1.15). In conclusion, different proportions of GABAergic interneurons, particularly calretinin neurons, reflect the structural and functional differences between cortical regions.

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PP9

REGULATION OF BROWN ADIPOSE TISSUE ACTIVITY BY BRAIN UROGUANYLIN

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Postprandial activation of brown adipose tissue (BAT) is gender and age dependent. Since the uroguanylin (UGN), as an agonist of guanylate cyclase C (GC-C), leads to browning after its prolonged i.c.v. application and is released from the gut after a meal, in this study we determined the acute activation of BAT by UGN. In this study, male and female C57Bl/6NCrl mice were used. The activity of BAT was determined by infrared thermography (FLIR T-1020). The expression of UGN in hypothalamus upon insulin or GLP-1 stimulation was determined by GUCA2B ELISA Kit. GC-C was localized in POMC and dopaminergic neurons in Arcuate nucleus of hypothalamus by immunohistochemistry. In older animals five time smaller amount of UGN i.n. significantly increase BAT activity when compared to i.p. application. This activation was smaller in female animals in diestrus and not present in estrus. Insulin and GLP-1, 2h after i.n. application decreased pro-UGN expression in hypothalamus. Differences in BAT activation due to estrous cycle could be explained by increased and different pattern of expression of GC-C in hypothalamus in female mice in diestrus. Application of insulin or GLP-1 decreases UGN expression in hypothalamus. When insulin or analogues of GLP-1 are used in treatment of diabetic patients, the decrease of BAT activity and glucose expenditure by BAT, via decrease of UGN expression, could be expected. This study may contribute to development of medicaments for activation of BAT for treatment of hyperglycaemia in diabetic patients, which will improve glucose metabolism and postpone insulin application.

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P2 Molecular neuroscience

PP10

METHYLATION PATTERNS OF DKK1, DKK3, SFRP4, AND GSK3B ARE ACCOMPANIED WITH DIFFERENT EXPRESSION LEVELS IN HUMAN ASTROCYTOMA

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We investigated genetic and epigenetic changes and protein expression levels of negative regulators of Wnt signaling, DKK1, DKK3, SFRP4 and APC as well as GSK3β and β-catenin in 64 human astrocytomas of grades II–IV. Methylation-specific PCR revealed promoter methylation of DKK1, DKK3, SFRP4 and GSK3β in 38%, 43%, 16% and 18% of samples, respectively. Grade IV comprised the lowest number of methylated GSK3β cases and highest of DKK3. Methylation of SFRP4 was present exclusively in grade II astrocytomas (72.8%) and appeared significantly more often than in grade III ($p=0.022$) and grade IV ($p<0.001$). Evaluation of the immunostaining using H-score was performed for β-catenin, both total and unphosphorylated (active) forms. Additionally, active (pY216) and inactive (pS9) forms of GSK3β protein were analyzed. Prevalence of β-catenin's active form ($rs=0.634$, $p<0.001$) was confirmed in astrocytoma tumor cells. We revealed that astrocytoma with higher levels of the active pGSK3β-Y216 had lower expression levels of its inactive form ($p<0.0001$, $Z=-5.332$). Changes in APC's exon 11 were observed in 44.44% of samples by PCR/RFLP. Astrocytomas with changes of APC had higher H-score values of total β-catenin compared to the group without genetic changes ($t=-2.264$, $p=0.038$). Furthermore, a positive correlation between samples with methylated DKK3 promoter and the expression of active pGSK3β-Y216 ($rs=0.356$, $p=0.011$) was established. Our results emphasize the importance of methylation for the regulation of Wnt signaling. Large deletions of APC associated with increased β-catenin levels, together with oncogenic effects of both β-catenin and GSK3β, are clearly involved in astrocytoma evolution. Further studies should elucidate the clinical and therapeutic relevance of the observed molecular alterations.

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PP11

TBR1 AS A KEY INDICATOR FOR CORTICAL PLATE CELLS SPREAD DOWN DURING THE PRIMATE CHARACTERISTIC SUBPLATE EXPANSION PERIOD IN THE HUMAN FETAL CORTEX

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Transient fetal zones are a characteristic feature of the developing human cerebral cortex. Neurons are born in the proliferative zones: ventricular (VZ) and subventricular zone (SVZ). Afterward, newly born neurons migrate through the intermediate zone (IZ) and subplate (SP) to reach their final destination, the cortical plate (CP). Development of the cortical anlage begins with the formation of preplate – PP (mantle by His), which is split by the cohort of CP cells in that way the specific marginal zone (MZ) containing Cajal-Retzius neurons (CRN) and presubplate (P-SP) are formed. During the early fetal period, P-SP is already present below the cortical plate, while the expanded SP is formed between 13 PCW and 15 PCW (SP formation period) as a key event in the corticogenesis of humans and other primates. Cells of the deep CP are secondarily dispersed downwards (second cortical plate), forming the future expanded SP. Tbr1 is a transcription factor highly expressed in the preplate and CP, and later in the SP and deep CP, suggesting its major role in cortical development. Reelin is a glycoprotein secreted by CRN in the MZ, playing a major role in neuronal radial migration. Our study aimed to show the expression dynamics of Tbr1, a marker of future SP neurons, and Reelin, during the early and midfetal period with the special emphasis on the SP formation period. We showed that Tbr1 is a reliable marker of CP cells` „displacement“ and final laminar destination during the human specific key event of corticogenesis – SP formation period.

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PP12

MALIGNANT TRANSFORMATION OF GANGLIOGLIOMA TO ANAPLASTIC GANGLIOGLIOMA: A GANGLIOSIDE CHARACTERIZATION STUDY

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Anaplastic gangliogliomas (AGGLs) are rare, neuronal-glial types of tumors classified as WHO grade III, strongly associated with a short overall survival rate. AGGLs are composed of dysplastic ganglion cells and anaplastic glial components. Although AGGLs occasionally occur de novo, benign gangliogliomas (WHO grade I) can develop a malignant transformation into AGGLs. Gangliosides are sialylated glycosphingolipids highly abundant in neural cells where they act as functional and structural components of a cell membrane. Changes in their composition are recognized as a hallmark of malignant transformation and they strongly correlate with tumor growth and invasiveness. Herein, we present a rare case of transformation from a low malignancy ganglioglioma to AGGL and characterization of ganglioside expression in AGGL, peritumoral (PT) and normal brain (NB) tissue. Complex ganglioside mixtures were isolated and purified from AGGL, PT and NB tissue in parallel. The total ganglioside content was spectrophotometrically determined and ganglioside fractions were characterized by a high performance thin-layer chromatography (HPTLC). The total ganglioside content in AGGL was approximately six times lower than in NB tissue and five times lower than in PT tissue. The ganglioside pattern of HPTLC-separated fractions showed a highly altered composition of ganglioside species in AGGL compared to PT and NB tissue. The ganglioside composition in PT tissue was highly similar to NB tissue. The ganglioside composition in AGGL was highly distinguishable from both PT and NB tissue. The ganglioside characterization elucidates a realistic state of ganglioside expression at the cell surface identifying the major ganglioside species acting as cell-surface antigens of specific tumor specimens.

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PP13

CHARACTERIZATION OF HUMAN TAU PROTEIN EXPRESSED IN QUIESCENT YEAST

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of tau protein aggregates in cells of the affected brain regions and the consequent neuronal death. In healthy neurons, tau is a soluble, intrinsically disordered protein that is mostly bound to the microtubules in the axon, however, in the affected neurons, tau accumulates in the soma and dendrites within amyloid-like aggregates, presumably via an intermediary step involving toxic oligomeric structures. The main risk factor for the onset of AD is aging, however, despite a vast number of studies, the causes of tau protein aggregation and toxicity are still largely unclear. Since the ability of a cell to maintain protein homeostasis decreases with aging, impaired protein quality control pathways are considered a possible factor in the development of AD. Yeast is a single cell eukaryotic organism that is amenable to genetic analysis, and many of its cellular pathways, including protein quality control are evolutionarily conserved. Moreover, quiescent yeast can be used to study processes characteristic for non-dividing cells, such as chronological aging. To investigate factors that influence tau protein aggregation and toxicity, in particular cellular aging and proteotoxic stress, we expressed human tau protein in yeast Saccharomyces cerevisiae. We will present a characterization of human tau protein expressed in quiescent yeast.

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PP14

TRANSCRIPTION FACTOR CUX2 IS EXPRESSED IN THE INTERNEURONS OF TRANSIENT CELLULAR COMPARTMENTS OF THE DEVELOPING HUMAN NEOCORTEX

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CUX2 is a transcription factor expressed in the neurons of differentiated upper cortical layers. CUX2 expression pattern throughout human fetal cortical development is mostly associated with pyramidal neurons of excitatory origin. CUX2 role in rodents is associated with synaptic development and molecular specification, while its role in human neocortical development is mostly unknown. Experiments on rodents already found that Cux2 is expressed in cortical interneurons that invade the pallium via tangential migration routes. Herein, we focused on the molecular characterization of CUX2 neuronal populations within the transient cellular compartments and possibly exploring its role during neurodevelopment. CUX2 protein co-expression with different neurotransmitter markers (NPY, GAD65/67, SST, nNOS) and calcium-binding protein calbindin is identified using immunofluorescence on formalin fixed paraffin-embedded sections of postmortem human brains from 8 to 25 post-conceptional weeks (PCW) of the Zagreb Neuroembryological Collection. In the earliest fetal stage, a co-expression of CUX2 and calbindin is present, while CUX2 expression in the early stages is somewhat weak and there is no visible overlap with GAD65/67 expression. At 13 PCW, no co-localization of CUX2 nuclei and SST or GAD65/67 markers was identified neither in MZ nor in pSP. During the midgestation, NPY co-localized with CUX2 nuclei both in MZ and SP in the neocortex at 21 PCW. Furthermore, we found a co-localization between SST and CUX2 nuclei in the SP, but not in the MZ. nNOS neurotransmitter is expressed and co-localized with CUX2 in some SP cells, even though it is not expressed in the MZ. GAD65/67 expression is visible both in the MZ and SP cells, yet without the overlap with CUX2 positive nuclei. We showed CUX2 expression in the population of interneurons within the transient fetal compartments throughout the cortical development. Besides, we have already found CUX2 expressed in the postmigratory, the earliest differentiated neuronal population of the two transient cortical compartments: SP and MZ. CUX2 expression in postmigratory SP and MZ neurons, as well as the SP somatostatin neuronal population of the human fetal cortex, suggests a role for CUX2 in the formation of the first neuronal circuits.

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PP15

DEFICIENCY OF B-SERIES GANGLIOSIDES IS ASSOCIATED WITH GREATER ACTIN DENSITY IN HIPPOCAMPAL TISSUE

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Animal cell glycocalyces consist of cell-specific ratios of different glycoconjugates, primarily gangliosides in the vertebrate nervous system. Gangliosides are sialylated glycosphingolipids synthesized by a set of glycosyltransferases and sialyltransferases in the Golgi apparatus by sequential addition of saccharide moieties to a lactosylceramide backbone. Knocking out sialytransferase St8Sia1 gene causes the absence of the b-series gangliosides (GD3, GD1b and GT1b). Interestingly, reduced nerve and myelin thickness has been observed in the peripheral nervous system of mice lacking GD3 during fetal development. Brains of six-month-old knockout (St8Sia1-/-) mice were compared to age-matched wild-type mice via several staining methods to determine the differences in hippocampus lipidomics, vascular surface area and actin composition. In comparison to knockout mice, wild-type mice showed significantly higher actin filament staining through the CA1, CA2 and CA3 area suggesting a role of GD1b and GT1b in actin assembly ($p<0.05$). Stronger cholesterol staining was observed in CA1 and CA2 of the knockout mice compared to the wild type, as well as a stronger phospholipid staining in the CA2 region of the hippocampus ($p<0,05$). Knockout mice showed a smaller total vascular surface area (alkaline phosphatase staining) in the CA3 region compared to the wild type ($p<0,05$) while mitochondrial biomarker succinate dehydrogenase was not affected. Our results indicate that deficiency of b-series gangliosides due to St8Sia1 gene knockout potentially led to disruption of the membrane anchors of cytoskeletal cell structures in the hippocampal region of the mouse brain with a potential effect on neuroplasticity.

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PP16

THE EFFECT OF GANGLIOSIDE COMPOSITION ON ACTIVITY, EXPRESSION AND SUBMEMBRANE LOCALIZATION OF PLASMA MEMBRANE CALCIUM ATPASE ISOFORMS IN MOUSE BRAIN

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Gangliosides are sialic acid-containing glycosphingolipids that reside in the outer leaflet of the plasma membrane, with their localization being more robust in lipid rafts (LR). Gangliosides are known to modulate the structure, function and localization of membrane proteins, including the plasma membrane Ca^{2+} ATPase (PMCA), thus having an impact on various signaling pathways including the plasma membrane Ca2+ ATPase (PMCA). PMCA plays an important role in the maintenance of levels of intracellular Ca^{2+}, essential to the functioning of neurons, with its activity and expression varying during aging and neurodegeneration. In this study we aimed to investigate the effect of ganglioside composition on PMCA activity, expression and submembrane localization of PMCA isoforms, using *St8sia1* null mice with impaired synthesis of gangliosides. Wild type (WT) and null mice littermates were sacrificed, brain tissue neuroanatomically dissected and cortical and cerebellar homogenates prepared. PMCA activity was measured spectrophotometrically. Protein expression of PMCA 1, 2 and 4 isoforms in homogenates was analyzed by Western blotting. LR and non-raft (nLR) fractions from cortices and cerebella were isolated by ultracentrifugation in discontinuous sucrose gradients, and submembrane localization of PMCA isoforms analyzed by Western blotting. Data revealed statistically lower PMCA activity in cortices of null mice compared to the WT mice. Total protein amount of PMCA1 and PMCA4 isoforms was statistically lower in cortices of *St8sia1* null mice compared to WT mice, whilst the expression of all three isoforms was unvaried in the cerebella. Analysis of submembrane localization was shown not to be statistically different between cortical LR and nLR fractions of null and WT mice, albeit there is statistically lower amount of all three isoforms in cerebellar LR fractions of null mice compared to their WTs. These results led us to hypothesize that alterations in ganglioside composition might contribute to loss of either PMCA activity or expression, related to ageing and neurodegeneration.

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PP17

TOLL-LIKE RECEPTOR 2 DEFICIENCY AFFECTS NEUROPLASTIN AND P-TYPE ATPASES EXPRESSION IN MOUSE BRAIN

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Transmembrane proteins, neuroplastin (Np), and Toll-like receptor 2 (TLR2) are implicated in tuning the neuroarchitecture. Np isoforms (Np55 and Np65) are related to synaptic plasticity and neuronal ion homeostasis by interactions with plasma membrane Ca2+ ATPases (PMCA). TLR2 is mainly expressed on microglia, but a much broader cellular localization of TLR2 has been established, particularly during neurodevelopment. To test the hypothesis that TLR2 deficiency affects membrane dynamics, we analysed membrane protein phenotype in the brains of TLR2 knock-out (KO) mice. Cortical, cerebellar, and hippocampal tissue derived from male TLR2-KO and age-matched control (C) mice (N=36+36) underwent systematic biochemical profiling encompassing: analysis of Np55, Np65, PMCA, Na+/K+-ATPase (NKA) expression by Western blot (WB); transcriptional variations of selected genes by qPCR; synaptic proteome by mass spectrometry (MS); activity of NKA and PMCA by spectrophotometry. MS analysis revealed astonishing differences in the synaptic proteome. Increased Np55, Np65, and NKA expression in cortex, cerebellum, and hippocampus was determined by WB in TLR2 vs. C. These findings were confirmed at a transcriptional level using qPCR. WB analysis of 4 PMCA isoforms showed that lack of TLR2 is associated with decreased expression of PMCA2 in cortex and cerebellum and increased in hippocampus. Changes in NKA and PMCA activity were also observed in TLR2 KO vs. C. Multiple-level analysis revealed that TLR2 deficiency leads to altered expression of proteins associated with synaptic plasticity and ion homeostasis. Observed proteomic changes are related to glutamatergic transmission, neuronal cytoskeleton organization, and mitochondrion energy metabolism. Further investigation may clarify previously undescribed roles of TLR2 in neuron-microglia interactions, synaptic connections arrangement, and neurotransmission.

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PP18

CONDURITOL B EPOXIDE AFFECTS INSULIN SIGNALLING IN DIFFERENTIATED SH-SY5Y CELL LINE

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Conduritol B epoxide (CBE) is a sugar derivative commonly used for experimental induction of Gaucher disease type 1. The compound acts by irreversible blocking of glucosylceramidase enzyme (E.C.4.2.1.25) thus preventing lysosomal degradation of glycosphingolipids and consequently results in glycosphingolipid accumulation. Gangliosides are a major group of glycosphingolipids affected with this treatment. Previously it was described that GM1 and GM3 in excess impair insulin signaling thus producing insulin resistance. SH-SY5Y human neuroblastoma cell line was differentiated with 10 µM of retinoic acid and treated upon differentiation in 48 hours duration with three different concentrations of CBE 2.5, 10 and 40 µM respectively. Additional groups were challenged with 2 µM/ml of insulin for 1 hour before proceeding with further experimental procedures. Cell viability was determined with MTT test. Activation of insulin signaling pathway was determined with Western blot method and changes in lipid metabolism was assessed with MALDI-TOF-MS. CBE had no significant effects on cell viability tested with MTT test regardless of concentration applied and insulin treatment. The application of high concentrations of CBE caused a significant threefold increase in pAkt levels compared to the untreated control group and a twofold increase compared to the medium and low concentration treated groups. The same pattern was observed in all insulin-treated groups compared to the untreated control. MALDI-TOF-MS analysis revealed down-regulation in sphingosine and cholesterol biosynthesis. Additionally, an accumulation of N-acetylneuraminate was observed when the highest concentration of CBE and insulin were applied.

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PP19

LAMINAR DYNAMICS OF CELF1 EXPRESSION IN EARLY AND MID FETAL HUMAN CEREBRAL WALL

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Precise regulation of gene expression is a prerequisite for normal brain development. Unlike regulation of transcription, which has been studied for decades, the importance of regulating mRNA translation during prenatal brain development has been studied only recently. Here we studied laminar dynamics of CELF1 expression pattern in prenatal human cerebral wall, as it was previously shown that Celf1 is required for the development of early neuronal progenitors and glutamatergic neurons during the mouse corticogenesis [1]. Dynamics of CELF1 expression in human brain is less known. The development of the prenatal human brain is characterized by the appearance of temporary fetal zones essential for the establishment of the proper organization of the future cerebral cortex. The dynamics of brain development, time course, and spatial organization are controlled by the regulation of gene expression at several levels. A number of factors are involved in posttranscriptional and translational regulation, including RNA-binding proteins (RBPs). The role of RNA-binding proteins is to control gene expression by creating ribonucleo-protein complexes. RBPs are involved in the regulation of neurogenetic processes, such as proliferation, cell specification, migration, and neuronal maturation [2]. Therefore, we analyzed CELF1 expression pattern utilizing immunofluorescence throughout prenatal postmortem human brain tissue in order to reveal laminar shifts and molecular specification of major classes of neurons. Our results showed the expression dynamics of RNA binding protein CELF1 during the early and mid fetal period. In addition we performed CELF1 double labelling with the upper cortical layer-enriched markers such as SATB2, CUX1, CUX2 and the lower cortical layer-enriched markers, such as TLE4, CTIP2, SOX5, TBR1. Accordingly, our results suggest CELF1 involvement in various neurogenetic processes during human corticogenesis such as neuronal identity, their molecular specification and laminar destination.

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PP20

TRIOBP-1 FORMS AGGREGATES WITH OTHER PROTEINS IMPLICATED IN MAJOR MENTAL ILLNESS

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Disrupted proteostasis and protein co-aggregation is a novel approach in studying underlying mechanisms of non-genetic onset of chronic mental illnesses and schizophrenia specifically. Here we studied the ability of Trio-Binding Protein 1 (TRIOBP-1), implicated as aggregating in schizophrenia, to recruit other proteins to co-aggregate by overexpressing them in neuroblastoma cells. It was our aim to determine whether TROBP-1 co-aggregates with other proteins implicated in mental illness (DISC1, CRMP1 Sv, NPAS3), and to discover if TRIOBP-1 can induce aggregation of its normal interaction partners. Our results show that overexpressed, aggregating wild type TRIOBP-1 recruits NDE1 to co-aggregate. Furthermore, TRIOBP-1 co-aggregates with DISC1, another protein implicated in mental illness, but not with CRMP1 Sv or NPAS3. Mutated TRIOBP-1 stabilises DISC1, while on the other hand DISC1 recruits mutated TRIOBP-1 to co-aggregate. We conclude that the co-aggregation of TRIOBP-1 is a highly specific process and potentially of significant relevance to mental illness. These newly discovered proteinopathies could serve as a potential biological markers and used for early schizophrenia diagnosis if discovered not to be limited to the brain. Protein co-aggregation may provide a valuable insight in underlaying mechanism of non-genetic schizophrenia onset.

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PP21

FOOTPRINT ASSAY - EVALUATION OF THE DIFFERENCE BETWEEN CHEMICALLY INDUCED AND CONGENITAL (B4GALNT-/-) DEMYELINATION OF MICE

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Disorders that lead to changes in the myelin sheath interfere with the normal conduction of electrical impulses and are manifested by various neurological symptoms. Changes in myelin sheath can occur by direct attack on the myelin or indirectly as a result of a primary genetic disorder, also by attacking oligodendrocytes during inflammation, or by exposure to toxic compounds. It has been shown that oral intoxication with copper-chelator cuprizone induces oligodendrocyte apoptosis, leading to demyelination and proliferation of glial cells, a major feature of neurodegenerative diseases. However, little is known about behavioral effect associated with cuprizone administration. Further, knocking out B4Galnt1 gene in mice blocks synthesis of complex gangliosides causing Waller's degeneration of axons, leading to demyelination of nerve fibers and impaired motor coordination. The aim of this study was to assess the impact of demyelination on motor coordination using two animal models - cuprizone model and B4Galnt1 knockout mice. The study was conducted on 8 groups consisting of 12 male mice three and 6 month old. The control group consisted of C57BL / 6N (wild-type, WT) mice and B4Galnt1-/- (KO), whereas other groups consisted of cuprizone fed WT and KO mice. Using the footprint assay we analyzed motor coordination and synchrony by examining gait during normal walking. Animals exposed to cuprizone showed more limp coordination deficits and motor impairment. This data together with ongoing immunohistochemistry and additional behavioral analysis will enable more detailed neurobiochemical characterization of chemically induced and congenital (B4Galnt-Null) demyelination, and give insight into functional implications on these findings.

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P3 Cognitive neuroscience

PP22

THE CORRELATIONS BETWEEN RESULTS IN DIFFERENT DOMAINS OF COGNITIVE ABILITIES MEASURED IN MEDICAL STUDENTS

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The aim of this study was to investigate the correlations between IQ test scores obtained using the Raven's Advanced Progressive Matrices (APM) and psychomotor testing using the Complex Reactionmeter Drenovac (CRD) test battery.In the period 2017-2019, 224 medical students at the University of Split School of Medicine studying in the English and the Croatian programs were recruited for this study. The IQ scores of the students were assessed using Raven's APM where students had to complete 36 items of the abstract reasoning test. The computerized test of CRD-series was used for testing reaction times of light stimulus perception (CRD311), operative thinking through complex psychomotor limb coordination (CRD411) and convergent thinking through solving simple arithmetic operations (CRD11). The total test solving time (TTST) and the minimum single task solving time (MinT) were analyzed. On the CRD11 test, task-solving times were significantly shorter in students with higher APM scores (r=-0.48 for TTST and r=-0.44 for MinT; P<0.001 for both tests). On the CRD311 test, there were also negative associations between task-solving times and APM scores (r=-0.30 for TTST and r=-0.33 for MinT, P<0.001 for both variables). The CRD411 test also demonstrated negative associations between task-solving times and APM scores (r=-0.40 for TTST and r=-0.30 for MinT, P<0.001 for both). In conclusion, this study demonstrated that achieving a higher APM score was associated with greater performances on the CRD-series reaction times tests, and therefore greater cognitive abilities for convergent thinking, operative thinking and speed of perception in medical students.

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PP23

WORKING MEMORY IN BLIND AND PARTIALLY SIGHTED CROATIAN ADOLESCENTS

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Cognitive abilities of blind and partially sighted children have been understudied in Croatia, and there is very little research on it. Since working memory plays quite an important role in cognitive functioning, the goal of this research was to explore the status of working memory in blind and partially sighted Croatian adolescents. To do so, 195 adolescents ages 12 to 16 completed the Wechsler Intelligent Scale for Children-IV (WISC-IV) at the Center for Education and Rehabilitation (CER) Vinko Bek. 75% of the participants were partially sighted, whereas the remaining 25% were blind. Males and females were represented in almost equal proportion. Participants' scores on the Working Memory Index (WMI) were analyzed and compared to other factors including the age of the onset of the visual impairment, additional developmental difficulties, and professional help. Our findings showed a significant low negative correlation between WMI and age of the onset of the visual impairment, suggesting that later onset is related to lower WMI score. Moreover, there were significant differences in WMI between groups with and without additional developmental difficulties. Professional help appears to have low positive effect on the WMI performance. These scores included performances on the following WISC-IV subtests: Digit Span, Letter Number Sequencing and Arithmetic. Results showed that there were no significant differences in working memory performances in between blind and partially sighted adolescents.

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P4 Neurodevelopmental basis of cognitive, mental and neurological disorders

PP24

EARLY VISUAL TRACKING BEHAVIOR IS LINKED TO THE VISIBILITY OF TRANSIENT BRAIN STRUCTURES IN PREMATURE NEONATES

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Premature infants often develop visual deficits, including difficulties in tracking behavior, even in the absence of ophthalmological complications and high-graded brain injury. Finding neuroimaging markers, especially considering subtle lesions, would allow the appropriate follow-up and intervention inducing mechanisms of brain plasticity, prominent at early age. Thus, more accurate information about nature of subtle perinatal lesions can be observed in spatiotemporal context of the white matter segments, precisely within the vulnerability of axonal pathways due to hypoxic-ischemic events. The aim of this study was to examine the link between visibility of 2nd white matter segment composed of sagittal strata (SS) and periventricular crossroads C1-C6 on MRI and early visual behavior in preterm neonates at term-equivalent age (TEA). 71 premature born infants (mean age=27+1 weeks of PMA) without high-graded brain injury on MRI and no ocular pathologies have been included in the study. The infants were MRI scanned at TEA, and brain injuries were classified as normal or mild according to the standardized MRI scoring system. The visibility of transient structural brain patterns (SS and periventricular crossroads C1-C6) was graded on a 3-point scale as being non-visible (1), poorly visible (2) or fairly visible (3) on MRI. At the median age of 42+5 PMA all infants completed the assessment of fixing and tracking movements (horizontally, vertically and in an arc) by Neonatal Visual Assessment. Difficulties in early visual tracking behavior were observed in 19 infants (27%). The deficits in tracking behavior were not related to the MRI classification. The analysis revealed significant correlation between tracking behavior and the visibility of crossroad C6. Furthermore, we have found strong correlation between tracking score and the visibility of frontal and occipital SS. Higher visibility of periventricular crossroads C6 and frontal and occipital SS on MRI performed at TEA correlated with better visual tracking skills in neonatal period. Our results once again confirmed sagittal strata and periventricular crossroads prominence as a valuable additional tool in perinatal neuroimaging at term-equivalent age. Those structures may have a significant role in risk prediction of visual functions deficits for very premature born infants with subtle or no evident brain injury.

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PP25

EXTRACELLULAR MATRIX PROFILE IN HIPPOCAMPAL SCLEROSIS

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Hippocampal sclerosis (HS) is the commonest histopathological finding in patients with drug-resistant mesial temporal lobe epilepsy (MTLE). The type 1 HS is the most prevalent type characterized by severe loss of pyramidal neurons, parvalbumin (PV) interneurons, and gliosis in the CA1 and CA4 fields. The extracellular matrix (ECM) is recognized as an important regulator of excitability and synaptic plasticity, especially in its highly condensed pericellular form of perineuronal nets (PNN). As experimental rodent models suggest that the different ECM components and PNN may have a role in epileptogenesis the aim of this study was to analyze and correlate PNN, glycosylation pattern with Wisteria floribunda agglutinin (WFA) and other ECM constituents, and expression of Nuclear-neuronal marker (NeuN), and parvalbumin (PV), with clinical findings of 65 patients surgically treated for pharmacoresistant MTLE caused by HS. Apart from the reduced number and impoverished morphology of pyramidal neurons and PV interneurons in HS, we found a changed distribution of the specific glycosylation pattern recognized by WFA. The proteoglycans, versican, and aggrecan show significantly changed expression patterns, whereas other ECM constituents as fibronectin, chondroitin-sulfate proteoglycan-56, and neurocan were slightly changed or showed no apparent difference. We also found reduced WFA specific glycosylation of the PNN around pyramidal neurons in all CA fields (CA4-CA1) as well as around PV-immunoreactive interneurons and simultaneously increased WFA specific glycosylation of the diffuse ECM in all CA fields. These findings suggest that PNN reduction and change of the glycosylation neuropil profile in the MTLE due to HS may cause the increase of the excitability of pyramidal hippocampal neurons, thus contributing to the development of drug-resistant epilepsy, at the same time offering a potential therapeutic target.

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P5 Sleep

PP26

METAMEMORY IN STUDENTS WITH NON-ORGANIC INSOMNIA

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Brain imaging techniques confirmed the involvement of the anterior and ventromedial prefrontal cortex, insula and cingular cortex in the process of metamemory. The role of these areas is not entirely clear, and it is assumed that the insula and cingular cortex provide input on cognitive performance, which the prefrontal cortex integrates into current goals and beliefs thus creating an introspective report of one's own memory. Research also confirm that people suffering non organic insomnia show reduced activity of the insula, medial prefrontal and cingular cortex which can ultimately affect their executive functions and impaired metamemory. Based on the above, the aim of the study was to examine the correlations between performance in declarative memory tasks and metacognitive assessments on group of university students who suffer from non organic insomnia and group who do not suffer from insomnia. Due to the possibility that metacognitive assessment is not adequately integrated into cognitive performance itself, no significant correlations are expected between performance in declarative memory tasks and metacognitive assessments in group of students with non organic insomnia. This group consisted of 52 participants who achieved a minimum score of 6 points on the Athens Insomnia Scale (criterion for non organic insomnia). The second group consisted of 48 students who did not meet the criteria for non organic insomnia. Both groups had no diagnoses of other sleep disorders or chronic diseases. For the purpose of this research, declarative memory task, which consisted of associative pairing of words, was constructed. Metacognitive assessments were given on a scale of 10% to 100%. The results show that both groups of students calibrated their own memory equally well, as evidenced by significant and high positive correlation between memory and metamemory. No significant differences in the size of correlation coefficients between the two groups were also found.

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PP27

COVID-19 LOCKDOWN REVEALED THE GREAT WORKLOAD, CHRONIC SLEEP DEPRIVATION AND ANXIETY OF MEDICAL STUDENTS IN THE PRE-LOCKDOWN PERIOD

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We aimed to investigate and compare differences and changes in sleep and lifestyle habits as well a mood of Croatian medical (MS) and non-medical students (NMS) during the COVID-19 lockdown. A cross-sectional study included 1163 participants (21.6% male) with median age 22 (IQR 20-23) whose demographics, teaching modes, lifestyle and sleep habits and mood before and during the COVID-19 lockdown were assessed with an online, self-reported questionnaire. Significant shifts toward later bedtimes and wake-times were reported in both MS and NMS (P<0.001), with the shift in bedtime being more pronounced among NMS (~65 min) compared to MS (~38 min), while the shift in wake-time was similar in both, MS (~111 min) and NMS (~112 min). All students reported more frequent difficulties falling asleep, night-time awakenings and insomnia (P<0.001) during lockdown. A higher proportion of MS reported being less tired during lockdown compared to pre-lockdown (P<0.001), which was not a case among NMS. Both students' groups experienced unpleasant moods (fear and discouragement) and were less content during lockdown compared to pre-lockdown period (P<0.001). There was a decrease in anxiety among MS (P<0.001), while NMS were more anxious (P=0.057) during lockdown. Sleep disturbances and unpleasant moods were more common among students, emphasizing the need for promotion of healthy habits in the youth population during lockdown. However, co-appearance of prolonged and delayed sleep time along with decreased tiredness and anxiety among MS during lockdown implies their significant workload during pre-lockdown and that even subtle changes in day schedule might contribute to well-being of MS.

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PP28

THE IMPACT OF AGE AND SEX ON BEHAVIOR AND MOOD CHANGES, SLEEP HABITS AND ATTITUDES TOWARD VACCINATION DURING THE COVID-19 PANDEMIC

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Our aim was to determine whether there were changes in behavior, mood and sleep habits during the lockdown caused by the SARS-CoV-2 virus depending on age and sex, and to analyze attitudes toward vaccination. During the two months (February 26 - April 26, 2021) of the COVID-19 pandemic, 636 people (74% women) completed an online questionnaire sent via social media, using a snowball method. Most respondents were resided in Croatia (62.26%), Bosnia and Herzegovina (29.72%) and Germany (6.92%). Demographic data, data on attitudes toward vaccination, and data on changes in behavior, mood and sleep habits before and during lockdown were collected. Overall respondents reported to exercise less and spend more time on social media during pandemic ($P < 0.001$). They went to bed and got up later during the lockdown. Women and respondents younger than 30 years had greater difficulty falling asleep and more frequent presence of insomnia ($P < 0.001$). Women and respondents over the age of 30 adhered better to pandemic measures. Slightly higher propensity to get vaccinated was shown by men compared to women, and respondents older than 30 years compared to younger ones. Women and respondents under the age of 30 were more prone to exhibit worsened mood during this pandemic ($P < 0.001$). Given that respondents' habits mostly deteriorated during lockdown and sedentary lifestyle has become even more pronounced, it is necessary to promote the benefits of regular physical activity, mental health care and the importance of sleep quality to get through this sensitive period trying to minimize negative long-term consequences.

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PP29

THE COVID-19 LOCKDOWN INDUCED CHANGES IN SLEEP HABITS AMONG CROATIAN GENERAL POPULATION

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The aim of the study was to investigate the effects of lockdown on sleep habits during the COVID-19 epidemic in the Croatian general population and to explore potential influence factors. Using an online cross-sectional survey, 1173 subjects from the general population (male=288, female=809) completed a self-reported questionnaire assessing demographic data, sleep habits and mood changes prior to and during lockdown. Results indicated a postponed bedtime (from 23:11±1:07 to 23:49±1:32 h, p<.001) and wake-time (from 6:51±1:09 to 7:49±1:40 h, p<.001) during lockdown. Sleep latency increased from 10 (5-20) to 15 (10-30) minutes during lockdown (p<.001). Bedtime and wake-time shift was more pronounced in females than in male subjects. Respondents under 30 years of age had the most pronounced delay in bedtime and wake-time. Also, they reported insomnia for the first time during lockdown more frequently and had less frequent awakenings (p<.001), less common problems falling asleep (p<.001), less frequently reported feeling calm (p<.001) and rested (p<.001), but expressed more frequent reports of sadness (p<.001) and fear (p=0.028) during lockdown than others. In conclusion, lockdown measures promoted a shift in sleep time along with prolonged sleep latency, increased first time reports of insomnia especially in respondents younger than 30 years of age, combined with mood changes. Younger age and less common problems with night-time awakenings and falling asleep were associated with increased odds for developing insomnia during the lockdown implemented during the COVID-19 pandemic.

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PP30

THE SLOPE OF THE OXYGEN DESATURATION AS A PREDICTOR OF ADHERENCE TO CONTINUOUS POSITIVE AIRWAY PRESSURE THERAPY IN SEVERE OBSTRUCTIVE SLEEP APNEA PATIENTS

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This study aimed to investigate the relationships of oxygen desaturation expressed as Slope Index (SI) and apnea severity expressed as Apnea-Hypopnea Index (AHI) with the adherence to Continuous Positive Airway Pressure (CPAP) therapy in severe Obstructive Sleep Apnea (OSA) patients. A retrospective study was performed on 70 severe OSA patients, with SI calculated as the averaged quotient of difference between the blood oxygen saturation level before and after the obstructive event (ΔSpO_2) and the duration of the obstructive event throughout the night, and AHI calculated as sum of apnea and hypopnea episodes per hour of sleep. CPAP usage for ≥4 hours/night on ≥70% of nights was defined as good CPAP adherence. Increased SI was associated with decreased percentage of nights with CPAP usage ≥4 hours during the first three months of CPAP usage (r=-0.314; p=0.008). No significant correlation was observed between AHI and percentage of nights with CPAP usage ≥4 hours during initial therapy period. The SI was significantly higher in patients with poor adherence during the first three months of CPAP usage in comparison to patients with good adherence (p=0.005). No difference in AHI was recognized between patients with good and poor adherence during the same period of CPAP usage. When logistic regression analysis was performed, a lower SI was a predictor of good CPAP adherence during the first three months of CPAP therapy (ß=0.004; p=0.019; R2=16.6%; p=0.010). Patients with a greater SI index or the slope of the oxygen desaturation curve were recognized as less likely to have good CPAP adherence following therapy onset. This novel parameter might give an insight on the impact of desaturation severity on CPAP adherence and might be valuable in recognizing patients with severe OSA at risk for poor CPAP adherence.

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PP31

THE ROLE OF ITEM T (TIREDNESS) IN THE STOP-BANG QUESTIONNAIRE AS A VALUABLE TOOL FOR ASSESSING THE RISK FOR OBSTRUCTIVE SLEEP APNEA

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Detecting Obstructive Sleep Apnea (OSA) early might contribute to a decreased public health economic burden. Especially when limited resources are available, low specificity might lead to unnecessary full-night recordings in healthy patients. The current study aimed to investigate predictive contribution of each item of the STOP-BANG questionnaire and pulse oximetry in OSA. Additionally, the role of older age was assessed. The study included 3128 participants admitted to Split Sleep Medicine Center, referred for polygraphy or polysomnography and assessed with STOP-BANG and Pittsburgh Sleep Quality Index (PSQI). Full-night polysomnography was conducted in-laboratory and full-night polygraphy was performed unattended. Following sleep assessment, 467 participants had no OSA and 2661 had mild to severe OSA. When only STOP-BANG variables were included in a model predicting confirmed OSA diagnosis, the largest amount of variability was explained in respondents <65 years old (R^2=35,7%; $p<0.001$). All predictors were significant with the exception of tiredness. Similarly, when mean saturation was included, the variance explained was also highest in respondents <65 years old (R^2=41,9%; $p<0.001$). Only tiredness and the diagnosis of hypertension were non-significant. In >65 year old respondents, lower predictive value was achieved in both models (R^2=19,9% and R^2=23,5%; $p<0.001$). When AUCs were calculated for correctly recognized OSA based on STOP-BANG as well as following the exclusion of item T (tiredness), STOP-BANG with the excluded item on tiredness performed the best. In conclusion, our findings indicated that the widely used STOP-BANG questionnaire as a valuable tool for assessing the risk for OSA might improve the overall predictive value if the item assessing tiredness (T) was excluded.

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PP32

DISRUPTED SLEEP IN BRAIN TUMOR PATIENTS - A NEGLECTED PROBLEM

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Malignant brain tumors are among the most aggressive human neoplasms, causing significant morbidity and mortality. Sleep disruption is one of the most common symptoms which malignant brain tumor patients experience. It is also one of the symptoms which impairs their quality of life the most. However, sleep evaluation and treatment is still unfortunately seldom considered and performed during current neuro-oncological treatment. There is a wide variety of systemic changes to an organism caused by disrupted sleep. These include phase shifts, reduced anti-oxidant levels, immunosuppression, various metabolic changes, melatonin depletion, cognitive impairment and epigenetic changes. All of these effects are in turn known to cause significant pro-tumor effects enabling further tumor progression. Here, we review the connection between disrupted sleep and the tumor progression, focusing on malignant brain tumors. We also review the hypothetical ways in which brain tumors actively disrupt sleeping schedules of patients making them especially susceptible to this kind of pathology. In conclusion, we argue that addressing the issue of disrupted sleep in patients with malignant brain tumors can possibly improve the quality of the patients' life and even have at least some potential of actively suppressing the devastating disease. This is especially true when other treatment modalities have been exhausted. Thus, future research into the relationship between malignant brain tumors and disrupted sleep are desperately needed.

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P6 Clinical neuroscience

PP33

ATTITUDES OF CROATIAN CITIZENS ON APPROACHES TO CHILDREN WITH DISABILITIES - ARE THERE DIFFERENCES WITH REGARD TO SOCIODEMOGRAPHIC CHARACTERISTICS?

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Considering that attitudes towards different approaches to the inclusion of children with disabilities are an important prerequisite for social change in terms of their equality, this study focused on capturing Croatian citizens'; agreement with three predominant approaches to this group: the medical model, the charity model and the social model of human rights. The main objective of the study was to examine whether citizens of the Republic of Croatia differ in their attitudes towards these models in relation to basic socio-demographic characteristics. The research was conducted on a representative sample of the adult population of the Republic of Croatia (N = 600) via telephone survey. Participants indicated the extent to which they agreed with the three statements describing the three theoretical approaches under study. The results showed that the citizens of the Republic of Croatia do not differ in their attitudes towards the three approaches, neither in terms of the six residential regions nor in terms of the settlement type (village vs. city). Moreover, agreement with the social model of human rights does not depend on gender, age, level of education and having own children. However, the results show that women and participants who have their own children are on average more likely to agree with the charity model and the medical model. The level of agreement with the charity model and the medical model also depends on age and education level. The level of agreement with the above two approaches is higher among older citizens and middle-aged citizens than among younger citizens, and higher among citizens with a lower level of education than among citizens with a higher level of education. The conducted research has shown that the social model of human rights is widely accepted, which indicates that the citizens of the Republic of Croatia are very aware of the rights and needs of children with disabilities.

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PP34

SOME CORRELATES OF MOTIVATION TO CHANGE IN FEMALE SUBJECTS WITH ANOREXIA NERVOSA

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Dosadašnja istraživanja skreću pozornost na nalaz da su osobe sa dijagnozom anoreksije nervoze slabije motivirane za liječenje, što je u konačnici povezano s nepovoljnim ishodima i visokim stopama odustajanja od liječenja. U srži motivacijskog deficita je ambivalencija glede želje za oporavkom, a koja se nerijetko povezuje sa skupinom oboljelih od ovog poremećaja. Preciznu identifikaciju trenutka spremnosti na promjenu maladaptivnog odgovora na životne okolnosti omogućuje transteorijski model namjerne promjene ponašanja. Ovaj cirkularni model pretpostavlja postojanje pet stadija kroz koje pojedinac prolazi u promjeni određenog ponašanja (predkontemplacija, kontemplacija, priprema, akcija i održavanje), a posebno je relevantan za ponašanja koje karakterizira motivacijski deficit, poput različitih ovisnosti. Cilj ovog istraživanja bio je ispitati povezanost motivacije za promjenom s intenzitetom različitih simptoma poremećaja hranjenja na uzorku od 33 sudionice koje pate od anoreksije nervoze u dobi od 20 do 33 godine. U online istraživanju je primijenjen Upitnik stadija promjene anoreksije nervoze (Rieger, Touyz, Schotte i sur., 2000) te Inventar poremećaja hranjenja (Garner, 1991) koji obuhvaća 8 simptoma koji se odnose na psihopatologiju poremećaja hranjenja, ali i na generalno psihološko funkcioniranje: težnja za vitkošću, nezadovoljstvo tijelom, bulimija, perfekcionizam, neučinkovitost, interoceptivna svjesnost, nepovjerenje u druge ljude i strah od odrastanja. Dobiveni rezultati korelacijskih analiza su pokazali da sudionice s nižom motivacijom za promjenom doživljavaju izraženije simptome poremećaja hranjenja, a riječ je o težnji za vitkošću, nezadovoljstvu vlastitim tijelom, strahom od odrastanja, neučinkovitosti i nepovjerenju u druge ljude. Nadalje, bulimija, interoceptivna svjesnost te perfekcionizam se nisu pokazali značajnim korelatima motivacije za promjenom. Najveći postotak sudionica (N=15; 45,50%) u trenutku provedbe istraživanja nalazio se u stadiju održavanja, dok se preostalih 18 sudionica (54,54%) nalazilo u stadijima predkontemplacije, kontemplacije, pripreme i akcije. Ove dvije skupine sudionica značajno su se razlikovale u određenim simptomima poremećaja hranjenja. Sudionice u stadijima predkontemplacije, kontemplacije, pripreme i akcije izvještavaju o doživljavaju značajno većeg straha od odrastanja te iskazuju značajno veće nepovjerenje u druge ljude u odnosu na skupinu sudionica u stadiju održavanja. Dobiveni rezultati ukazuju da bi se sudionice s anoreksijom, koje manifestiraju veću motivaciju za promjenom, mogle vrednovati kao potencijalna skupina za postizanjem povoljnijih ishoda u tretmanu u odnosu na skupinu s nižom motivacijom za promjenom, što se može i očekivati sukladno postavkama transteorijskog modela namjerne promjene ponašanja.

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PP35

STRUCTURAL CHANGES IN BRAINS OF PATIENTS WITH DISORDERS OF CONSCIOUSNESS TREATED WITH DEEP BRAIN STIMULATION

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Disorders of consciousness (DOC) are one of the major consequences after anoxic or traumatic brain injury. So far, several studies have described the regaining of consciousness in DOC patients using deep brain stimulation (DBS). However, these studies often lack detailed data on the structural and functional cerebral changes after such treatment. The aim of this study was to conduct a volumetric analysis of specific cortical and subcortical structures to determine the impact of DBS after functional recovery of DOC patients. Five DOC patients underwent unilateral DBS electrode implantation into the centromedian parafascicular complex of the thalamic intralaminar nuclei. Consciousness recovery was confirmed using the Rappaport Disability Rating and the Coma/Near Coma scale. Brain MRI volumetric measurements were done prior to the procedure, then approximately a year after, and finally 7 years after the implementation of the electrode. The volumetric analysis included changes in regional cortical volumes and thickness, as well as in subcortical structures. Limbic cortices (parahippocampal and cingulate gyrus) and paralimbic cortices (insula) regions showed a significant volume increase and presented a trend of regional cortical thickness increase 1 and 7 years after DBS. The volumes of related subcortical structures, namely the caudate, the hippocampus as well as the amygdala, were significantly increased 1 and 7 years after DBS, while the putamen and nucleus accumbens presented with volume increase. Volume increase after DBS could be a result of direct DBS effects, or a result of functional recovery. Our findings are in accordance with the results of very few human studies connecting DBS and brain volume increase. Which mechanisms are behind the observed brain changes and whether structural changes are caused by consciousness recovery or DBS in patients with DOC is still a matter of debate.

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PP36

DEEP BRAIN STIMULATION IN DISORDERS OF CONSCIOUSNESS: A 10 YEAR INSTITUTIONAL EXPERIENCE

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Disorders of consciousness, namely vegetative state (VS) and minimally conscious state (MCS), represent serious conditions with mayor consequences for patients and their families. Several studies have described regaining of consciousness in such patients using deep brain stimulation (DBS) of brain or brainstem nuclei. The aim of our study is to present results of 10 years' experience in using DBS as a therapy for VS/ MCS patients. Overall, 63 patients were included in the study. Entry criteria included neurophysiological and neurological evaluation, as well as neuroimaging examination. DBS system was implanted in 26 patients; 20 patients were in VS and 6 in MCS. The stimulation target was centromedian-parafascicular complex in the left hemisphere in HI-BI or more preserved one in TBI patients. Follow up ranged from 10 to 111 months. Level of consciousness was improved in six patients. Two MCS patients regained consciousness, ability to walk without any help, to speak fluently and without need for everyday life assistance. Four patients improved to the level of consciousness with possibility of non-verbal communication, but are still dependent on the care of their guardians. In patients with disorders of consciousness a spontaneous recovery to the level of consciousness without assistance in everyday life is very rare. Thus, for such patients that fulfill neurological, neurophysiological and neuroimaging criteria, DBS of certain thalamic nuclei could be advised and recommended as a treatment option, especially in earlier phases when irreversible changes of musculoskeletal system are not so expressed.

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PP37

FRAMELESS STEREOTACTIC BRAIN BIOPSY: A PROSPECTIVE STUDY ON ROBOT-ASSISTED BRAIN BIOPSIES PERFORMED ON 32 PATIENTS BY USING THE RONNA G4 SYSTEM

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We present a novel robotic neuronavigation system, RONNA G4, used for precise preoperative planning and frameless neuronavigation. The system has been developed by a research group from the University of Zagreb and a team of neurosurgeons from the University Hospital Dubrava, Zagreb, Croatia. The aim of this study is to provide a comprehensive error measurement analysis of the system used for brain biopsy procedures. Frameless stereotactic robot-assisted biopsies were performed on thirty-two consecutive patients. Post-operative computerized tomography (CT) and magnetic resonance imaging (MRI) scans were assessed to precisely measure the target point error (TPE) and the entry point error (EPE). All clinical data, the learning curve, and the influence of the trajectory angle on targeting accuracy were measured and evaluated. The application accuracy of the RONNA system for the TPE was 1.95 ± 1.11 mm, while for the EPE was 1.42 ± 0.74 mm. In our cohort, only one pathohistological diagnosis was inconclusive; thus, the total diagnostic yield was 96.87%. Linear regression showed statistical significance between the TPE and EPE and the angle of the trajectory on the bone ($p=0.026$, $p=0.010$). The learning curve analysis showed statistical significance, especially for one neurosurgeon who performed most of the procedures ($p<0.001$). The operation duration was significantly reduced over time, as shown by comparing the first ten procedures with the last ten procedures ($p=0.0007$). According to the results of our comprehensive analysis of TPE and EPE, the RONNA G4 robotic system is a precise and highly accurate autonomous neurosurgical assistant in performing frameless brain biopsies. Greater trajectory angles were associated with larger EPE and TPE.

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PP38

IMPROVING ELECTROPHYSIOLOGICAL CRITERIA FOR IMPLANTATION OF DEEP BRAIN STIMULATION IN PATIENTS WITH DISORDERS OF CONSCIOUSNESS: A PRELIMINARY STUDY

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Disorders of consciousness (DOC) are one of the major consequences after anoxic or traumatic brain injury. Several studies have described the regaining of consciousness in DOC patients using deep brain stimulation (DBS). Prior to DBS implantation, both electrophysiological and clinical criteria should be fulfilled. Thus, the aim of this study was to perform extended electrophysiological testing in order to establish improved electrophysiological protocol prior to DOC DBS. Five DOC patients underwent electrophysiological criteria in order to assess the indication of DBS implantation under propofol sedation. Multimodal evoked potentials such as somatosensory evoked potentials (SEP), motor evoked potentials (MEP), brain stem auditory evoked potentials (BAEP) and electroencephalography (EEG), as well as additional, the electrical response of the cerebral cortex, P250 potential and high-frequency SEP oscillation thresholds (600 Hz) were analyzed. Presence of SEP, MEP and BAEP regardless of pathological parameters indicated adequate cortical response. The P250 showed to be potential that emerges from giving stimuli so that the respondent feels pain with a latency of 200 to 300 ms. The amplitudes of high frequency SEP oscillations higher than 70 nV showed good prognostic sign, while amplitudes with the values below suggested severe anoxic encephalopathy incompatible with the return of consciousness. Spontaneous recovery to the level of consciousness in DOC patients is very rare. Thus, for such patients it is necessary to define electrophysiological criteria for DBS implantation as possible. Although our study is preliminary, the presented data are extremely important due to possibility to exclude severe anoxic injury, and thus predict the clinical outcome. Electrophysiological criteria, disorder of consciousness, CM-pf, vegetative state, minimal consciousness state.

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PP39

PROTECTIVE FACTORS OF MENTAL HEALTH DURING THE SECOND LOCKDOWN IN CROATIA

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Pandemija COVID-19 je izazvala brojne promjene u odvijanju svakodnevnoga života, ali i dovela do pojave različitih teorija zavjera vezanih uz novi koronavirus odnosno bolest koju izaziva. Dosadašnja istraživanja sugeriraju kako viši stupanj vjerovanja u COVID-19 teorije zavjera predviđa lošije mentalno zdravlje, ali i niži stupanj poštivanja epidemioloških mjera. Cilj ovoga istraživanja bio je ispitati odnos sociodemografskih karakteristika, poštivanja epidemioloških mjera, vjerovanja u COVID-19 teorije zavjera i mentalnog zdravlja. Pripremljeni online upitnik sadržavao je sociodemografska pitanja, Skalu COVID-19 teorija zavjera, Skalu zavjereničkog mentaliteta, Kratki upitnik mentalnog zdravlja te Upitnik pridržavanja epidemioloških mjera. Istraživanje je provedeno od studenog do prosinca 2020., a prikupljena su 793 odgovora. Utvrđene su spolne razlike u poštivanju epidemioloških mjera, vjerovanju da je virus napravljen od strane ljudi, zavjereničkom mentalitetu te mentalnom zdravlju. Preciznije, sudionice su se više pridržavale epidemioloških mjera u odnosu na sudionike, a stupanj vjerovanja da je virus napravljen od strane ljudi te zavjereničkog mentaliteta također je bio viši kod sudionica u odnosu na sudionike. Sudionici su bili boljeg mentalnog zdravlja u odnosu na sudionice. Provedbom hijerarhijske regresijske analize uz kontrolu sociodemografskih obilježja utvrđeno je kako su značajni prediktori mentalnog zdravlja muški spol, viša dob te pripadnost desnom političkom spektru. Rezultati ovoga istraživanja sugeriraju važnost pristupa provjerenim, pouzdanim i točnim izvorima informacija kako bi se zaštitilo mentalno zdravlje.

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PP40

NEUROSCIENTIFIC ASPECTS OF GAMBLING DISORDER AMONG ADOLESCENTS

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Risky behavior associated with a gambling disorder occurs already in adolescence, thus 5 % of adolescents are problem gamblers. Poor mental and physical health, financial problems, family conflicts and deviant behavior are gambling disorder consequences. Interaction between psychosocial factors and neurodevelopmental vulnerability of the adolescent brain are the main risk etiological factors. If there is a constant participation in a gambling activity, e.g. due to peer pressure, the risk of developing problem gambling behavior is higher. However, adolescence is a critical period of vulnerability for problem gambling as the brain motivation structures are being developed, thus adolescents with the same amount of time spent in gambling develop more serious disorders than adults. Most developmental changes occur in the dopamine neuronal system, which the motivation neuronal circuit consists most of. Furthermore, the number of synapses in the prefrontal cortex increases in this period. However, there is a synaptic reduction of synapses located in the local circuits in order to save energy for distant synapses that affect multimodal processes, including motivation. The maturation of motivational circuits at the end of adolescence facilitates the motivation development for activities that lead to delayed but greater gratification. Until this prefrontal cortex maturation is complete, adolescents are more susceptible to the problem gambling development, because they find it harder to delay prompt and immediate gratifications. Since the problem gambling, for the reasons described, occurs early in adolescence, it is necessary to develop comprehensive preventive and therapeutic programs specific for this group to prevent gambling disorder.

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PP41

VARIANT NERVE FIBERS ORGANIZATION IN BRACHIAL PLEXUS FORMATION

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The brachial plexus (C5-T1) is a network of nerve fibers organized in trunks, cords and major terminal branches without communicating branches being standardly described. Major terminal branches of the brachial plexus include the median, musculocutaneous, ulnar, radial and axillary nerve. In this study we have carefully dissected and analyzed 58 upper extremities from 29 formaldehyde-preserved cadavers. Variations from the pattern of the brachial plexus defined in anatomical textbooks were observed in 67% limbs. The most observed group of variations (74%) are additional communicating branches from one of the cords towards the opposite cord, root, or branch. The second group of observed variations (21%) are communications between the musculocutaneous and median nerve. The number of observed variations creates doubt whether anatomy typically described in textbooks is truly standard anatomy. Moreover, two specific, not previously described cases were found: one in which the lateral cord is formed exclusively from the upper trunk; and another, in which the anterior division of the middle trunk is completely included in the formation of the medial root of the median nerve. These findings raise the question whether fibers passing from the lateral cord to the medial are in fact fibers from the C7 spinal segment, and in which amount does the C7 spinal segment even participate in musculocutaneous nerve formation. Presence of variations not fitting in any commonly used classifications suggests potential need for modifying existing classifications, as there is a great clinical significance related to axillary surgery and differential diagnosis of peripheral neuropathies.

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P7 Hypoxic-ischemic damage

PP42

EPIGENETIC AND GENETIC MODIFICATIONS OF THE GM1 GANGLIOSIDE EXPRESSION LEVELS TO STUDY THE NEURORESTORATIVE PROPERTIES OF THE MOLECULE AFTER AN ISCHEMIC LESION OF THE MOUSE BRAIN

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Ischemic stroke is one of the main causes of death worldwide caused by a loss of blood flow to the brain resulting in cerebral hypoxia and irreversible neuronal damage with activation of the ischemic cascade highly dependent on the duration of the hypoxic state and the affected brain area. There is a potential for use of ganglioside GM1 in ischemic stroke therapy based on its neuroprotective properties resulting in the promotion of neural stem cells, suppression of oxidative stress, and cell death. Previous studies and clinical trials have shown that intravenous or intrathecal application of ganglioside GM1 could be beneficial in ischemic stroke animal models and patients with its limitations. Epigenetic and genetic modifications will increase the expression level of the GM1 ganglioside molecule after an ischemic mouse brain lesion. With the help of mouse models with reduced expression of the enzyme G2 synthase to study the neurorestorative properties of GM1 gangliosides. Male St8siaI mice and corresponding wild-type animals as controls. To induce ischemic stroke, the anesthetized animal will undergo the MCAO method. Behavioral changes will be observed in Digital Ventilated Cages (DVCs) for 24-hour continuous monitoring. The animals will be sacrificed 3 and 7 days after the ischemia. Collected brain tissue will be rapidly frozen on dry ice for molecular analysis or fixed in 4% paraformaldehyde, cryoprotected in a sucrose gradient, and frozen in cold isopentane for immunohistochemical analysis. The magnitude of the stroke will be determined by MRI and the functional consequences by behavioral and neurological status tests. The ganglioside GM1 could potentially be neuroprotective in cerebral ischemic conditions and its therapeutic properties are being considered for ischemic stroke management. Currently available KO mice with a complete lack of GM1 ganglioside present an opportunity to investigate and demonstrate the effect of this ganglioside in ischemic stroke recovery which may have important implications in clinical practice in terms of ganglioside administration itself (intrathecally) or effects on its synthesis.

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PP43

POST-ISCHEMIC REACTIVATION OF THE CORTICOGENESIS MARKERS BCLL11B AND SATB2 IN THE MOUSE BRAIN

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The aim of the study was to investigate activation of the corticogenesis markers BCL11B and SATB2 after ischemic lesion of the adult mouse brain. The study was conducted on 3 months old male transgenic mice C57BL/6-Tyrc-Brd-Tg(NFH-luc/turboFP635)Gaj. One group (n=6) underwent a 60 min middle cerebral artery occlusion (MCAO) followed by reperfusion and the other group (n=4) was sham operated. Mice were imaged by high resolution magnetic resonance imaging (MRI) before the surgery and 3 and 7 days after MCAO. Seven days after the surgery brains were isolated and immunohistochemistry was preformed using markers of corticogenesis (BCL11B, SATB2) and markers of brain plasticity after ischemia (ATF3, HDAC2). Results were quantified by cell counting and by optical density calculation. The size of ischemic lesion was the largest 3 days after MCAO. Immunohistochemistry showed increase of BCL11B and SATB2 signal in cortex, striatum and hippocampus 7 days after ischemic lesion of the mouse brain, compared to sham operated animals. Moreover, increase in co-localization of BCL11B and SATB2 after brain ischemia was observed in the cortex and striatum. Markers of brain plasticity after ischemia ATF3 and HDAC2 had higher expression in MCAO group, compared to sham operated animals, and their co-localization with BCL11B was observed in the cortex and striatum. In conclusion, corticogenesis markers BCL11B and SATB2 had higher expression after ischemic lesion of the mouse brain. Co-localization of BCL11B with ATF3 and HDAC2 indicated increased expression of corticogenesis markers after ischemia was related to the processes of brain plasticity after ischemia.

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PP44

NOVEL STATISTICAL MODEL ADAPTED FOR MULTIMODAL ASSESSMENT OF BRAIN LESION RECOVERY AFTER INDUCED ISCHEMIC STROKE

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So far, due to large variability in outcomes following induced ischemic stroke in mice, single modality approaches have shown to be underpowered and therefore lacking in ability to detect potential novel therapies with moderate or low effect sizes. Our goal was to construct a comprehensive statistical model that encompasses various modalities in hopes that it would bring us a novel and robust approach for future use. Two groups of animals, wild-type(WT) and TLR2 -/- mice, have been compared at multiple time points. Transient medial artery occlusion (tMCAO) was done by introducing a filament through internal carotid artery and by retracting it after 60 minutes to allow for both the ischemic and reperfusion injury of the brain. Brain MRI scans were used to derive brain lesion sizes and edema index values. Modified neurological severity score was used to assess sensory and motor functions. Mice were also weighted to account for possible confounding effect. All of statistical modeling was done in R Studio environment. Analysis of variance for repeated measures did not show any significant difference between groups on both lesion size and neurological score data. Using cumulative linear mixed-modeling (CLMM) as a method of ordinal regression we managed to explain 59% of variance in neurological scores by including relative stroke volume in the model. Both the stroke size and the group allocation significantly determined neurological score, with $P<0.01$ on both variables. Many in vivo experiments regarding stroke suffer from lack of statistical power in data analysis. Multimodal approach could prove essential in tackling those challenges. It is our belief that it is a viable post-hoc approach for overcoming both limited numbers in group sizes and aforementioned variability in data collected. Multimodal statistical analysis for assessing stroke shows a significant improvement over conventional approaches commonly used in literature so far.

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PP45

A SYSTEMATIC REVIEW OF PRECLINICAL IN VIVO STUDIES ON VIRUS-MEDIATED GENE THERAPY IN ISCHEMIC STROKE

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A few therapeutic approaches are currently available for ischemic stroke. Lentivirus (LV) and adeno-associated virus (AAV) mediated gene therapy has been extensively investigated in preclinical stroke studies for successful gene delivery. Based on the search of three databases, our systematic review provided the current state of LV and AAV mediated gene interventions in preclinical ischemic stroke models. The search analyzed studies published till January 2021 and identified 87 relevant studies further, which were further analyzed qualitatively and quantitatively. Data regarding animal and stroke model, viral vector characteristic, administration method and outcome measures were used. The results showed that the preferred stroke model was MCAO, performed mainly on adult male rodent animal models. Both AAV and LV vectors were used equally for transgene delivery driven in the most cases by a constitutive promoter. Viral vector delivery was performed mostly before the stroke induction via stereotaxic injection in the cortex and striatum. Overall, risk of bias was high, while methodological and result reporting was insufficient. The meta-analysis based on infarct volume analysis as the primary outcome confirmed the therapeutical potential of viral vector gene delivery. Our results indicated that viral vectors represented safe and effective method of therapeutical gene delivery to ischemic stroke animal models. However, the improvements such as higher standardization of study design and reporting, post-stroke viral vector mediated gene delivery and non-invasive delivery method could increase the translational potential of this intervention from the preclinical research to the clinical setting.

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PP46

THE RELATIONSHIP BETWEEN TLR2-MEDIATED INFLAMMATION AND APOPTOSIS AFTER ISCHEMIC LESION OF THE MOUSE BRAIN

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Innate immune response after ischemic stroke is characterised by long-term induction of Toll-like receptor 2. However, it is unclear whether the induction of immune response after ischemia is protective or detrimental. Our hypothesis was that the reduction in immune response after ischemia increased apoptosis. The aim of this research was to measure the scope of apoptosis after ischemic stroke in vivo. In this study, wild type and Tlr2-/- mice with ubiquitous luciferase expression were used. Neurological deficiency scoring, bioluminescence and magnetic resonance imaging were conducted for all mice at baseline, 2, 7, 14 and 28 days post ischemic stroke. Ischemic brain lesion was induced with transient middle cerebral artery occlusion. Apoptosis was measured using bioluminescence with caged Z-DEVD-aminoluciferin and immunohistochemistry staining. Prior to bioluminescence imaging, a dose-response experiment with caged Z-DEVD-aminoluciferin was performed on transgenic mice with ubiquitous luciferase expression. On day 2 post stroke, Tlr2-/- mice had significantly less tissue edema than the wild type controls. On days 14 and 28 post stroke, Tlr2-/- suffered a statistically significant larger loss of ipsilateral tissue compared to wild type controls. Tlr2-/- animals had better survival. The dose-response study of Z-DEVD-aminoluciferin indicated that the optimal concentration for in vivo imaging is 10 mg/kg. Differences in total bioluminescence flux were not significant between groups. The significance in edema on day 2 post-stroke in Tlr2-/- mice compared to the wild type controls could be explained by a weaker immune response to stroke induction in Tlr2-/- mice. Significantly larger ipsilateral tissue loss in Tlr2-/- mice on days 14 and 28 post-stroke suggested that there is a mechanism by which normal inflammation preserves the tissue. Better survival of Tlr2-/- animals possibly suggested that smaller initial lesion volumes and reduced inflammation and its following effects contribute to survival through the chronic post-stroke period.

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PP47

EFFECT OF UROGUANYLIN ON DEVELOPMENT OF ISCHEMIC BRAIN LESION

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Stroke is one of the leading causes of mortality and disability in industrialized countries. Guanylate cyclase (GC) A activation has a neuroprotective effect after ischemic stroke therefore the aim of this study is to determine if agonists of GC-C have similar effects. Uroguanylin (UGN) activates guanylate cyclase C (GC-C) and a Ca^{2+} -dependent signaling pathway. In this study, middle cerebral artery occlusion (MCAO) was performed on wild type (WT), GC-C KO and UGN KO mice. Before and 24h after MCAO MR images were taken. 48h following MCAO brain slices were isolated and Ca^{2+} response to UGN stimulation was recorded. Immunohistochemical staining was performed with GC-C, NeuN, and GFAP antibodies. WT and UGN KO animals exhibit a stronger Ca^{2+} response to UGN stimulation in astrocytes of the ischemic penumbra in cerebral cortex but not in the unaffected hemisphere. This stronger activation is gone in GC-C KO animals which results in development of smaller ischemic lesions in GC-C KO mice compared to their WT littermates. Considering the fact that GC-C becomes expressed on penumbral astrocytes following ischemia, while in normoxic conditions it is expressed only in cortical neurons, effects of GC-C on intracellular Ca^{2+} concentration could be due to activation of cGMP-dependent Ca^{2+} channels in penumbral astrocytes. Stronger activation of the Ca^{2+}-dependent signaling pathway could lead to the development of larger ischemic lesions in WT compared to GC-C KO animals, possibly through upregulation of Na^{+}/H^{+} exchanger followed by tissue acidification and neuronal death.

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PP48

LIGHT SHEET FLUORESCENCE MICROSCOPY IN THE ASSESSMENT OF STRUCTURAL CHANGES IN THE MOUSE BRAIN AFTER ISCHEMIC LESION

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Ischemic stroke is one of the major health problems in the modern society. It occurs due to the blockage of blood vessels in the brain, which interrupts nutrients and oxygen supply to the brain. Modern neuroimaging techniques provide insight in lesion localization and volume, but not in the cellular structure and the morphological correlates of the damage and repair. Novel light sheet fluorescence microscopy (LSFM) allows to visualize large 3D objects, which can be used on whole mouse brain in order to determine cell morphology and morphological changes after stroke. Achieving high optical transparency of the sample is a crucial step before using LSFM. The aim of this study was to establish tissue clearing method that provides good transparency for acquiring 3D image of the whole mouse brain using LSFM. Acquired images would allow for 3D reconstruction and could subsequently be correlated with other neuroimaging methods (magnetic resonance imaging and in vivo bioluminescence imaging). Combination of these methods paves the path for better understanding of ischemic stroke by multimodal approach. Generally, three types of tissue clearing techniques can be distinguished: organic-solvent-based, aqueous-based and hydrogel-based. Brains of adult wild type and Gap43-GFP transgenic mice were isolated and cleared using two organic-solvent-based methods: PEGASOS and iDISCO. Tissue transparency was estimated using grid drawn on a piece of paper. Brains were photographed after each tissue clearing step. Both blurriness and shrinkage of cleared brain tissue were less noticeable when using PEGASOS method. Autofluorescence was visualised on cleared brain slices using EVOS microscope. Brains cleared with PEGASOS method show satisfying transparency and could potentially be used for 3D image acquisition on LSFM, however the autofluorescence was present as well.

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PP49

PRECLINICAL APPROACHES TO ADDRESS THE BRAIN RECOVERY AFTER ISCHEMIC STROKE

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Acute therapy of ischemic stroke includes thrombolysis or thrombectomy and there are no other targeted therapies for patients, only symptomatic therapy and rehabilitation which may or may not be successful. Therefore additional research and understanding of the functional recovery process is necessary. The goal of this research is to get insight into the functional recovery of mice after ischemic stroke. Longitudinal recovery of mice will be monitored by recording their activity in their home cages 24/7, assessing modified neurological severity score, behavioral outcomes, and kinematic movement analysis. The functional outcomes will be correlated with recovery from brain magnetic resonance imaging. A clinically relevant model of ischemic stroke corresponding to ischemic stroke in humans treated with either thrombolysis or thrombectomy will be performed on mice by the transient middle cerebral artery occlusion followed by reperfusion. The mice subsequently suffer a functional sensory-motor deficit manifested by gait and posture disorders. Digital ventilated cages (Tecniplast, IT) measuring animal activity will be used for home-cage monitoring. This way of monitoring the activity allows insight during the night when the mice are active. Kinematic movement analysis will be performed using a MotoRater (TSE, DE) and recordings by high speed camera to evaluate 4 different motion modalities – overground walking, skilled ladder walking, wading in water and swimming. Kinematic gait analyses conducted with MotoRater are the method of choice to compare rodent gait with human conditions. The results of this study will facilitate the assessment of the long-term outcomes of the animal's recovery and to attempt to distinguish actual recovery from compensatory mechanisms.

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PP50

BRAIN AND RETINAL ISCHEMIA-REPERFUSION INJURY IN DIABETIC MICE WITH BRADYKININ TYPE 2 RECEPTOR DEFICIENCY

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Diabetes mellitus (DM) increases cerebral and retinal susceptibility to ischemic events and exacerbates the ischemic damage. Activation of the bradykinin signaling pathway plays a detrimental role in the acute phase of cerebral and retinal ischemia-reperfusion injury. The aim of this study was to determine the effects of DM on the acute phase of brain and retinal ischemia in bradykinin type 2 receptor (B2R) deficient mice. Male diabetic C57BL/6-Ins2Akita/J (Akita) mice, diabetic B2R knockout B6.Cg-Ins2Akita/Bdkrb2tm1Jfh/SmiJ (Akita/B2R-KO) mice and their non-diabetic controls (WT and B2R-KO) underwent a 30-minute middle cerebral artery occlusion (MCAO) to induce simultaneous brain and retinal ischemia. Seven days before, and on the 1st and 3rd day after MCAO the animals were subjected to neurological scoring, fundus photography, fluorescein angiography and MRI. There were no differences in survival or lesion size between groups, however diabetic Akita/B2R-KO mice showed increased swelling of the ipsilateral hemisphere with pronounced neurological deficit compared to non-diabetic controls. Neither diabetic nor non-diabetic B2R deficient mice showed edema resolution on the 3rd day, which was pronounced in the Akita and WT groups. On the 1st day after MCAO all groups showed significant thickening of the chorioretinal layer of the ipsilateral eye with severe necrosis and partial loss of the capillary network. On the 3rd day, the chorioretinal swelling persisted only for the non-diabetic B2R-KO while it partially reverted towards baseline levels in Akita/B2R-KO mice. Presence of DM modifies in opposite fashion the acute ischemic injury in the B2R deficient brain and retina.

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PP51

THE LONG-TERM EFFECTS OF BRADYKININ RECEPTOR TYPE 2 DEFICIENCY IN A MURINE MODEL OF CEREBRAL AND RETINAL ISCHEMIA

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Bradykinin is one of the key inflammatory factors released during brain and retinal ischemia, exerting both detrimental and protective actions across the course of the ischemic injury development. Since previous studies failed to elucidate the exact long-term effects of bradykinin receptor type 2 (B2R) activation, we aimed to clarify the role of B2R in the brain and retinal ischemia by longitudinal in vivo magnetic resonance (MR) assessment. To induce simultaneous ischemic injury of the brain and retina, 4 months old male C57Bl/6J and C57BL/6J/Bdkrb2tm1Jfh/SmiJ (B2R KO) mice underwent a transient 30-minute middle cerebral artery occlusion (MCAO) by filament insertion. Seven days prior and 2, 9, and 35 days following MCAO we performed neurological scoring and 7T MR imaging of the brain and the ipsilateral eye. Animals were sacrificed on the 35th day, the brains and eyes were isolated and processed for histological analysis. MR data analysis showed that the MCAO induced formation of cortico-striatal lesions in both groups, characterized by edema formation in the acute phase and severe tissue loss in the chronic phase, which was significantly more pronounced for B2R KO. The changes in measured chorioretinal thickness correlated with the progression of brain ischemia, showing thickening 2 days after MCAO and chorioretinal thinning 35 days after MCAO. This study showed that the B2R deficiency leads to poorer recovery and significant brain tissue loss compared to control animals, which indicates a protective role of B2R activation in the chronic phase of the ischemic brain injury.

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PP52

LIFELONG CHANGES AFTER PERINATAL MODERATE HYPOXIA: INCREASED NUMBER OF INTERNEURONS AND PERINEURONAL NETS IN THE CINGULATE CORTEX OF THE ADULT RAT

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We designed a non-invasive model in newborn rats to determine the hallmarks of possible permanent structural and behavioral changes in the brain after moderate perinatal hypoxia. The 82 Wistar Han (RccHan: WIST) rats were randomly subjected to hypoxia (pO2 73mmHg/2h) or normoxia at the first postnatal day. The increase of hypoxia-inducible factor-1α and decreased cytochrome-C-oxygenase expression in treated animals' brains, detected by Western blot, confirmed an acute moderate lesion. The results of the open-field, hole-board, social-choice, and T-maze tests, applied at the 30-45th and 70-85th postnatal days, displayed significant hyperactivity and a slower pace of learning in rats subjected to perinatal hypoxia. At 3.5 months of age, the histochemical examination revealed a significantly increased number of specific extracellular matrix-perineuronal nets and parvalbumin-expressing interneurons in the medial and retrosplenial cingulate cortex of these animals. Conclusively, moderate perinatal hypoxia in the rat causes lifelong cellular and connectivity organization changes in the cingulate cortex and related behavioral and cognitive alterations. This non-invasive hypoxia in the rat successfully and complementary models the frequently unrecognized moderate perinatal brain injury in fetuses in humans and may enhance future research of new diagnostic and therapeutic strategies for perinatal brain injuries.

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PP53

INCREASED DOPAMINE SIGNALING IN THALAMUS OF ADOLESCENT RATS NEONATALLY EXPOSED TO MILD NORMOBARIC HYPOXIA

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Mild hypoxic events during an early mid-gestation may cause subtle motor, sensory and behavioral deficits that become detectable only later in life, unabling so an early intervention. In order to search for early behavioral, structural, neurochemical, and molecular markers of mild hypoxia, we have developed a corresponding rat model by exposure of 1-day-old pups (PND1) to hypoxic conditions (8% O2, 92% N2) in a normobaric chamber for 2 hours. In adolescent rats, we have previously observed altered exploratory behavior, paralleled by midbrain dopamine (DA) increase, which was interpreted as possible difficulties in hippocampus (Hc)-related spatial mapping or thalamus (Th)-related somatosensory processing. This study examined possible alterations in DA signaling in the mentioned regions receiving inputs from the midbrain DA-neurons. By using qPCR, relative mRNA expression of D1 and D2 receptors, and their down-stream targets protein kinase A (PKA) and dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32), was measured in 16 hypoxia-exposed and 15 control rats sacrificed on PND50. In comparison to controls, relative D1 and D2 mRNA levels were unchanged in hippocampi but were significantly increased in thalami of the treated animals. Increased thalamic expression of D1 was accompanied by highly correlated upregulation of mRNA for PKA (regulatory subunit 2A) and DARPP-32, suggesting that one of the consequences of the neonatal exposure to hypoxia might be a long-lasting increase in the thalamic DA signaling. Further research on the thalamo-cortical signaling and somatosensory processing should reveal the contribution of this neuronal path to the observed behavioral changes.

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P8 Neurodegeneration

PP54

MAOB SINGLE NUCLEOTIDE POLYMORPHISM AS POTENTIAL GENETIC BIOMARKER OF ALZHEIMER'S DISEASE

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The normal functioning of dopaminergic system is compromised during Alzheimer's disease (AD). The activity of monoamine oxidase B (MAOB), enzyme involved in degradation of dopamine, is also disturbed in AD. It was observed that MAOB activity is increased during AD. Also, increased expression of MAOB was detected in hippocampus and cortex of people who suffered from AD. It was observed that MAOB rs1799836 polymorphism can affect MAOB transcription, consequently influencing also protein translation and MAOB activity. Our recent study showed that the levels of cerebrospinal fluid amyloid β1-42 were decreased in patients carrying A allele in MAOB rs1799836 polymorphism. The goal of this study was to compare MAOB rs1799836 polymorphism with APOE, the only confirmed genetic risk factor for sporadic AD. Study included 253 participants of whom 127 suffered from AD, 57 were mild cognitive impairment patients, 11 were healthy controls and 58 suffered from other primary causes of dementia. We observed that the number of APOE ε4/ε4 homozygotes and APOE ε4 carriers was significantly increased among patients carrying AA MAOB rs1799836 genotype. These results indicate that the MAOB rs1799836 polymorphism could be strong genetic biomarker of AD.

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PP55

CROSSTALK OF OPTINEURIN AND TDP-43 IN MICROGLIA IN THE BASAL STATE AND DURING INFLAMMATION

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Mutations in optineurin, an ubiquitin-binding adaptor protein implicated in various cellular functions including inflammatory signaling, protein trafficking, and autophagy, have recently been found in amyotrophic lateral sclerosis (ALS). ALS is a neurodegenerative disorder in which degeneration of motor neurons leads to progressive paralysis and death. Despite more than 50 genes mutated in ALS, the common hallmarks of ALS and other neurodegenerative diseases are chronic inflammation and protein aggregation. More than 95% of ALS patients have aggregated TAR DNA binding protein 43 kDa (TDP-43) in neurons and glia, whereby TDP-43 gets ubiquitinated, hyperphosphorylated, and mislocalized to the cytoplasm. Mutations in optineurin, although present in a small fraction of ALS patients, also cause aggregation and mislocalization of TDP-43, but the putative mechanistic link between optineurin and TDP-43 pathology is still elusive. To address this, we are using: (1) optineurin knockout (KO) neuronal (NSC-34 and N2A) and microglial (BV2) cell lines made by CRISPR/Cas9 technology, and (2) primary neurons and microglia from a mouse optineurin insufficiency model with truncation of the ubiquitin-binding region (Optn470T), similar to some mutations found in ALS patients. We found elevated basal TDP-43 protein levels in Optn KO BV2 microglial cell line and the primary Optn470T microglia. We did not detect differences in TDP-43 mRNA levels at basal state, arguing that TDP-43 is post-translationally regulated. Elevated TDP-43 protein levels were specific to microglia and were not observed in Optn KO NSC-34 and N2A cell lines, and primary Optn470T neurons. To test if inflammation could trigger TDP-43 aggregation in cells lacking functional optineurin, we stimulated them with lipopolysaccharide (LPS) to mimic bacterial infection. We observed a significant increase in TDP-43 levels in WT BV2 and primary microglia upon 2 different doses of LPS stimulation. On contrary, TDP-43 levels in BV2 KO and Optn470T microglia were not increased but remained at the same elevated state as in basal conditions. TDP-43 mRNA levels were not increased in Optn470T compared to WT microglia upon LPS, suggesting that LPS is not changing transcription of TDP-43. Autophagy blockade in both the basal state and upon LPS treatment did not lead to significant TDP-43 accumulation neither in WT and KO microglia cell lines nor in primary WT and Optn470T microglia, suggesting autophagosomal degradation in not a primary way of TDP-43 turnover in these cells. In conclusion, TDP-43 is increased in the basal state in microglia with optineurin insufficiency or deficiency, and unlike in WT cells, its levels do not rise upon LPS treatment. We hypothesize that lack of functional optineurin leads to chronically activated cells but further experiments are necessary to elucidate the crosstalk of these proteins in ALS pathogenesis.

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PP56

NEUROPROTECTIVE EFFECT OF BDNF IN AN IN VITRO MODEL OF ALZHEIMER´S DISEASE

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Alzheimer´s disease (AD) is a multifactorial neurodegenerative disorder still without effective and stable therapeutic strategies. It is characterized by the progressive cognitive decline and neuronal death, especially in the hippocampus and neocortex, which is probably due to the accumulation of beta amyloid (Aβ) plaques and neurofibrillary tangles. The etiology of AD is still not clear; however, there are many known genetic and environmental risk factors. Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophic factor in the adult brain, whose levels are reduced in AD. BDNF plays an important role in synaptic plasticity, synaptogenesis, neuronal survival and growth, particullary in the hippocampus, the brain region essential for learning and memory. This study has investigated potential neuroprotective effect of BDNF in primary neuronal cultures derived from the C57BL/6 mice. Primary mouse neurons were treated with various concentrations of toxic Aβ oligomer preparations, as well as with different BDNF concentrations. The colorimetric MTT assay, Promega cell-based and biochemical assays and Muse cell analyzer were used to investigate Aβ-induced cytotoxicity, as well as neuroprotective effects of BDNF. The results demonstrated that Aβ oligomers caused necrosis and apoptosis of neuronal cells, rather than oxidative stress, presumably acting via membrane integrity disruption, as well as caspase and bcl-2 activation. The observed neuroprotective effect of BDNF alone, but also in the combination with neurosteroids dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) is, at least partly, achieved by blocking the caspase activation and suggests beneficial antiapoptotic actions of BDNF on the survival of neurons damaged in AD.

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PP57

CIRCADIAN DYSRHYTHMIA IN A STREPTOZOTOCIN-INDUCED RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Circadian dysrhythmia has been recognized as a potential etiopathogenetic factor and an early sign of Alzheimer's disease (AD) in humans. We aimed to explore the patterns of circadian dysrhythmia in a streptozotocin intracerebroventricularly-treated (STZ-icv) rat model of sporadic AD (sAD). Adult male Wistar rats were treated icv with STZ (3 mg/kg) or vehicle (CTR) (nCTR=9, nSTZ=9; 3 animals per cage) and subjected to 11-day measurements of the motor activity patterns starting 3 days after the treatment. For a long-term acquisition of the spontaneous motor activity data, MIROSLAV (Multicage InfraRed Open-Source Locomotor Activity eValuator), a home cage-mounted system based on passive infrared sensors was developed based on passive infrared sensors, along with the appropriate supporting software. The circadian motor activity patterns were assessed using cosinor analysis. Cosinor analysis of the hourly sensor activation rate demonstrated a reduction in amplitude (A) in the STZ-icv group (ACTR=0.14, CI=0.11-0.17; ASTZ=0.06, CI=0.03-0.09; p=0.012) with unchanged MESOR (Midline Statistic Of Rhythm; M) values (MCTR=0.29, CI=0.18-0.40; MSTZ=0.29, CI=0.15-0.43; p=0.90), indicating a specific pattern of circadian dysrhythmia with reduced nocturnal, and increased diurnal activity in an early-stage experimental sAD. Preliminary findings indicate MIROSLAV may enable reliable long-term noninvasive monitoring of animals' circadian rhythm. Dysrhythmic motor patterns with fragmented sleep and reduced wakefulness observed in a STZ-icv rat model of an early-stage sAD resemble those reported in AD patients.

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NLRP1 AND ASC EXPRESSION IN ALZHEIMER'S DISEASE: CORRELATION WITH NEUROPATHOLOGICAL CHANGES IN THE HIPPOCAMPAL FORMATION

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Aberrant immune response has been implicated in the pathogenesis of Alzheimer's disease (AD), but it is not clear how it leads to neuronal dysfunction and cognitive decline. One of the possible mechanisms could be the overactivation of the inflammasomes. Those multiprotein complexes are mostly expressed in the myeloid lineage (including microglial cells), except the NLRP1 inflammasome that is primarily expressed in pyramidal neurons. Some polymorphisms of the NLRP1 gene have been associated with AD onset. We aimed to compare the expression of NLRP1 and ASC molecules in postmortem tissue of the different fields of the hippocampal formation in 12 AD and 12 control subjects. NLRP1 and ASC were visualized immunohistochemically. Semiquantitatively assessed NLRP1- and ASC-immunoreactivity was correlated with age, duration of the disease, and severity of characteristic neuropathological changes. Preliminary results will be presented and discussed during the poster session.

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TAR DNA-BINDING PROTEIN 43 REGULATION IN MICE SPINAL CORD DURING THE FIRST WEEK FOLLOWING REPETITIVE MILD TRAUMATIC BRAIN INJURY

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Repetitive mild traumatic brain injury (mTBI) is a risk factor for the development of different neurodegenerative diseases, including amyotrophic lateral sclerosis which is characterized by TAR DNA-binding protein 43 (TDP-43) proteinopathy in most cases. The aim of this research was to examine intracellular regulation of TDP-43 in the spinal cord during the first week after repetitive mTBI in mice. Repetitive mTBI was done by the weight drop method (Kane et al. 2012) twice a day, for five consecutive days in C57BL/6 mice. The control group of mice was subjected to a sham procedure, had the same handling and anesthesia, but received no impact. The mice were sacrificed one, three or seven days after the final injury/sham procedure and their spinal cords were dissected and prepared for the Western blot analyses of the proteins of the interest. In the spinal cords of injured mice, increased cytoplasmatic expression of the phosphorylated TDP-43 was detected on the first day after repetitive mTBI, while the expression of TDP-43 and its cleavage product TDP-35, as well as of the phosphorylated TDP-35 and TDP-25 were not impaired at any of the time points investigated. Our preliminary results suggest early and transient dysregulation of TDP-43 in the cytoplasm of the spinal cord cells in mice following repetitive mTBI, which are probably the result of an acute response to stress caused by the brain trauma.

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ROLE OF FERROPTOSIS IN WALLERIAN DEGENERATION OF GANGLIOSIDE DEFICIENT B4GALNT1 -/- MICE

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Mice of B4Galnt1 -/- genotype, which lack complex gangliosides, develop progressive Wallerian degeneration from a young adult age. As the gangliosides GD1a and GT1b are myelin-associated glycoprotein (MAG) receptors, the lack of their interaction at the axon/oligodendrocyte boundary is the current explanation for demyelination. But in maintaining the connection between axons and oligodendrocytes in B4Galnt1 -/- overexpression of 0-series gangliosides and GM3 serve as alternative receptors for MAG. It is not known why this compensation is not sufficient and what is the mechanism by which demyelination occurs. Using the imaging mass spectrometry, we compared the lipid composition in the corpus callosum of B4Galnt1 -/- (KO) and wild-type (WT) mice at the age before (3 months) and after the onset of demyelination (9 months). The principal component analysis (PCA) showed a 64.1% difference between KO and WT at 3 months of age and 76.1% of the difference at 9 months of age. The most of differentialy expressed compounds were upregulated in older KO animals. Four out of the twelve compounds with uniquely identified m/z ratio were classified within altered metabolic pathway of ferroptosis. Together with the finding of reduced activity of the mitochondrial enzyme succinate dehydrogenase, this speaks in favor of oxidative damage to oligodendrocytes as one of the reasons for their disfunction and death.

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PROTECTIVE EFFECT OF 20-HETE INHIBITION IN A MODEL OF OXYGEN-GLUCOSE DEPRIVATION IN N27 NEURONAL CELLS

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20-Hydroxyeicosatetraenoic acid (20-HETE), a potent vasoconstrictor, is a cytochrome P-450 (CYP)-derived metabolite of arachidonic acid. Inhibitors of 20-HETE synthesis and/or actions have been reported to protect brain from ischemic stroke in the rat and primate. The neuroprotective effects were not associated with an increase in cerebral blood flow suggesting that these drugs may enhance the survival of neurons after ischemic injury independent of their effect on cerebral blood flow. In this regard, our previous study has demonstrated that 20-HETE directly promote neuronal injury in organotypic hippocampal slices subjected to oxygen-glucose deprivation (OGD) and that inhibitors of 20-HETE synthesis protect neurons from OGD-induced cell death by decreasing ROS formation and activation of caspase-3. In line with our findings, protective effect of 20-HETE inhibition has also been reported in mouse cortical neurons after OGD. In the present study we used N27 rat dopaminergic neuronal cells subjected to OGD followed by reoxygenation to examine whether 20-HETE contributes to ischemic injury through activation of NADPH oxidase and increased superoxide production. The preliminary results indicate that cell viability after OGD increased after treatment with a 20-HETE synthesis inhibitor or an antagonist. Administration of a 20-HETE mimetic had the opposite effect and increased neuronal cell death after OGD. That effect was reversed by coadministration of a NADPH oxidase inhibitor, apocynin, suggesting that 20-HETE amplifies neuronal cell death by increasing oxidative stress through NADPH oxidase-dependent mechanisms. Further studies will be taken to elucidate the molecular mechanisms underlying the protective effect of 20-HETE inhibitors against OGD-induced neuronal injury.

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