



Fotoarhivo TZG Zadar

# 7<sup>th</sup> Croatian Neuroscience Congress

**Zadar - Croatia / 12<sup>th</sup> - 15<sup>th</sup> September, 2019**

University of Zadar, Obala kralja Petra Krešimira IV. 2

BOOK OF ABSTRACTS

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**BOOK OF ABSTRACTS**

## ORGANIZERS

- Croatian Society for Neuroscience (CSFN)
- Croatian Institute for Brain Research (CIBR)
- Centre of Excellence for Basic, Clinical and Translational Neuroscience
- Croatian Academy of Sciences and Arts, Department of Medical Sciences
- University of Zadar

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- Dario Nakić
- Benjamin Banai
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## ADDRESS OF THE ORGANIZER:

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Croatian Institute for Brain Research

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## GENERAL INFORMATION:

### CONGRESS VENUE

University of Zadar, Obala kralja Petra Krešimira IV. 2

### SCIENTIFIC PROGRAMME OVERVIEW

#### Presidential symposium „Current trends in neuroscience“

Speakers:

- **Paško Rakić** (Yale University, School of Medicine, USA);
- **Gundela Meyer** (La Laguna University, Tenerife, Spain);
- **James A. Barkovich** (UCSF Benioff Children's Hospital, USA);
- **Karoly Mirnics** (University of Nebraska Medical Center, USA);
- **Željka Korade** (University of Nebraska Medical Center, USA);
- **Mladen Roko Rašin** (Rutgers Robert Wood Johnson Medical School, USA);
- **Lana Vasung** (Harvard Medical School, USA);
- **Ivana Rosenzweig** (King's College London, United Kingdom);
- **Alan Antičević** (Yale University, School of Medicine, USA);
- **Ivana Dellale** (Boston Children's Hospital, USA)

#### Special lecture

Speaker:

- **Zoltan Molnar** (Department of Physiology, Anatomy and Genetics, University of Oxford)

#### Poster session topics:

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

#### Satellite events

- Presentation of the Centre of Excellence for Basic, Clinical and Translational Neuroscience, School of Medicine, University of Zagreb (**Miloš Judaš** and collaborators)
- Public lecture (**Nenad Šestan**, Yale University, School of Medicine, USA)

### Social events

- Welcome Cocktail
- Conference Dinner
- Excursion to Kornati National Park (optional)

### LANGUAGE OF THE CONFERENCE

The official language of the meeting will be English and Croatian.

### REGISTRATION FEES

	Early registration July 29th, 2019	Late and on-site registration after July 29th, 2019
<b>CSfN member</b>	<b>1.050 kn (≈ 145,00 €)</b>	<b>1.300 kn (≈ 175,00 €)</b>
<b>Non-member of CSfN</b>	<b>1.200 kn (≈ 165,00 €)</b>	<b>1.600 kn (≈ 220,00 €)</b>
<b>PhD student</b>	<b>450 kn (≈ 60,00 €)</b>	<b>450 kn (≈ 60,00 €)</b>
<b>One-day attendance</b>	<b>400 kn (≈ 55,00 €)</b>	<b>400 kn (≈ 55,00 €)</b>
<b>Accompanying person</b>	<b>550 kn (≈ 75,00 €)</b>	<b>550 kn (≈ 75,00 €)</b>

\*Fee prices are given in Croatian kunas.

#### Included in the registration fee:

Name tag; Congress material; Certificate of attendance; Welcome cocktail; Coffee breaks with refreshments; Conference dinner at Hotel Kolovare

Students are waived of the registration fee / Must fill online registration form / Conference dinner at Hotel Kolovare is not included in student registration.

### POSTER SESSIONS

Posters will be displayed on Saturday, 14<sup>th</sup> September, from 09.30 - 12.00 (Poster session I) and from 14.00 - 16.30 (Poster session II).

### POSTER PRESENTATION AWARD

Poster presentations are important scientific contributions, therefore a prize for the best poster presentations is established. It is awarded to the three best posters out of all poster sessions. 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.

### DOCUMENTS AND BADGES

Meeting documents should be collected on-site at the registration desk. The participants are kindly asked to wear the name tags during the Meeting and in the exhibition area.

## PROGRAMME

### Thursday, 12<sup>th</sup> September 2019

- 16.00-17.00 REGISTRATION OF PARTICIPANTS**
- 17.00-18.00** CoreNEURO Opening remarks  
(**Miloš Judaš, Marijan Klarica**)  
ROUNDTABLE DISCUSSION  
Centre of Excellence for Basic, Clinical and Translational Neuroscience, School of Medicine, University of Zagreb  
(**Miloš Judaš** and collaborators)
- 18.00-18.45 PUBLIC LECTURE**  
**Nenad Šestan:** Building the human brain: molecular logic of neural circuit formation
- 19.00** *Core NEURO Welcome cocktail*

### Friday, 13<sup>th</sup> September 2019

- 08.30-09.30 REGISTRATION OF PARTICIPANTS**
- 09.30-10.00 PRESIDENTIAL SYMPOSIUM "Current trends in neuroscience"**  
WELCOME ADDRESS & OPENING REMARKS
- 10.00-10.20** **Gundela Meyer:** The diversity of Cajal-Retzius neurons in the developing human cortex
- 10.20-10.40** **James A. Barkovich:** Disorders of cerebral cortical development – new concepts
- 10.40-11.00** **Mladen Roko Rašin:** Post-transcriptional events in developing neocortex
- 11.00-11.30** *Coffee break*
- 11.30-11.50** **Lana Vasung:** Quantitative *in vivo* MRI assessment of structural asymmetries and sexual dimorphism of transient fetal compartments in the human cerebral cortex
- 11.50-12.10** **Karoly Mirnics:** Neuronal cholesterol biosynthesis and homeostasis
- 12.10-12.30** **Alan Antičević/Lisa Jie Ji:** Characterizing neuro-behavioral geometry embedding via neuropharmacology, transcriptomics & clinical neuroimaging
- 12.30-14.00** *Welcome lunch / cocktail*

- 14.00-14.20** **Ivana Dellale:** GWAS-identified Alzheimer's disease risk variants – lessons from neuropathology
- 14.20-14.40** **Željka Korade:** Molecular bases of cholesterol biosynthesis disorders
- 14.40-15.00** **Ivana Rosenzweig:** Neurophysiology of the extraterrestrial sleep – possibility or culpability?
- 15.00-16.00** **Paško Rakić:** Building on 50 years of experience to shape my research today (and in the future)
- 19.00-23.00** *Conference dinner at Hotel Kolovare*

### Saturday, 14<sup>th</sup> September 2019

- 09.00-09.30 REGISTRATION OF PARTICIPANTS**
- 09.30-12.00 Posters and Discussions I**
- 12.00-12.45 PRESIDENTIAL LECTURE**  
**Zoltan Molnar:** Cortical layer with no known function
- 12.45-14.00** *Lunch break*
- 14.00-16.30 Posters and Discussions II**
- 16.30-17.00** Conference closing remarks and Best poster award

### Sunday, 15<sup>th</sup> September 2019

- 10.00-16.00** Excursion to Kornati National Park – optional

## POSTER PRESENTATIONS

### Saturday, 14<sup>th</sup> September 2019 - POSTER SESSION I

- 09.30 – 12.00 P1 BASIC NEUROSCIENCE  
P2 CLINICAL NEUROSCIENCE

### Saturday, 14<sup>th</sup> September 2019 - POSTER SESSION II

- 14.00 – 16.30 P3 COGNITIVE NEUROSCIENCE  
P4 MOLECULAR NEUROSCIENCE  
P5 NEURODEGENERATIVE DISORDERS  
P6 NEURODEVELOPMENTAL BASIS OF COGNITIVE, MENTAL AND NEUROLOGICAL DISORDERS  
P7 NEUROPHARMACOLOGY  
P8 SLEEP  
P9 HYPOXIC-ISCHEMIC DAMAGE

## LIST OF POSTERS

### Saturday, 14<sup>th</sup> September 2019 - POSTER SESSION I

#### 09.30 – 12.00 P1 BASIC NEUROSCIENCE

- PP1** RAPID GOLGI IMPREGNATION OF PYRAMIDAL NEURONS IN THE PREFRONTAL CORTEX IN SCHIZOPHRENIA  
*Banovac I, Sedmak D, Rojnić Kuzman M, Hladnik A, Petanjek Z*
- PP2** MORPHOLOGICAL ANALYSIS OF VON ECONOMO NEURONS  
*Banovac I, Sedmak D, Džaja D, Jalšovec D, Jovanov Milošević N, Rašin MR, Petanjek Z*
- PP3** SMARTPHONE OPHTHALMOSCOPY – A NOVEL METHOD FOR TRACKING CHANGES IN THE RETINA AFTER ISCHEMIC STROKE IN DIABETIC MICE  
*Barić A, Radmilović M, Justić H, Kežić J, Škokić S, Brkić A L, Dobrivojević Radmilović M*
- PP4** STRIATAL MEDIUM SPINY NEURONS SHOW GENDER DIFFERENCES IN DENDRITIC MORPHOLOGY  
*Bičanić I, Džaja D, Darmopil S, Petanjek Z*

- PP5** GROWTH OF HUMAN STRIATUM DURING FETAL AND EARLY POSTNATAL LIFE REVEALED BY VOLUMETRIC ANALYSIS  
*Blažević A, Raguž M, Sedmak D, Džaja D, Radoš M, Kostović I*
- PP6** DO SUBTHALAMIC AND SUBSTANTIA NIGRA NEURONS SHARE COMMON NEURONAL LINEAGE?  
*Bokulić E, Medenica T, Sedmak G*
- PP7** SYNAPTIC CHANGES WITHOUT SIGNS OF NEURODEGENERATION IN THE ROSTRAL CEREBRUM AFTER TRAUMATIC BRAIN INJURY IN THE RAT  
*Dolenec P, Pilipović K, Gržeta N, Župan Ž, Župan G*
- PP8** B4GALNT1-KNOCKOUT AND CUPRIZONE-INDUCED DEMYELINATION IS ASSOCIATED WITH CHANGES IN PARVALBUMIN AND CALRETININ-EXPRESSING INTERNEURONS IN THE MURINE CORTEX  
*Fenrich M, Viljetić B, Zjalić M, Habjanović K, Blažetić S, Heffer M*
- PP9** EXPRESSION ANALYSIS OF A NOVEL NEUROPEPTIDE, UROGUANYLIN, IN THE HUMAN PREFRONTAL CORTEX  
*Habek N, Sedmak D, Petanjek Z, Dugandžić*
- PP10** SEX SPECIFIC CHANGES IN EXPRESSION OF MAJOR BRAIN GANGLIOSIDES IN THE HIPPOCAMPI OF MIDDLE-AGED RATS ON WESTERN DIET  
*Ivić V, Rončević A, Labak, Zjalić M, Čosić A, Drenjančević I, Heffer M*
- PP11** INFLUENCE OF LEVEL OF OXYGEN ON EXPRESSION OF MAP1LC3A, A MARKER OF AUTOPHAGY  
*Jagečić D, Hribljan V, Lisjak D, Mitrečić D*
- PP12** SINGLE MODERATE TRAUMATIC BRAIN INJURY IN MICE CAUSES CHANGES IN PSD-95 IMMUNOREACTIVITY IN THE IPSILATERAL CORTEX AND HIPPOCAMPUS  
*Janković T, Gržeta N, Župan G, Pilipović K*
- PP13** CHALLENGES IN COMBINING IN VIVO AND EX VIVO VOLUMETRIC ANALYSIS OF MOUSE ISCHEMIC BRAIN  
*Justić H, Barić A, Skokić S, Dobrivojević Radmilović M, Dullin C, Tromba G, Filipović N, Mioc P, Gajović S*
- PP14** PROLIFERATIVE CAPACITY OF NEOCORTICAL PROGENITORS IS LINKED TO CHANGES IN THEIR MORPHOLOGY  
*Kalebic N, Huttner WB*
- PP15** CHLORPROMAZINE SPECIFICALLY AFFECTS EXPRESSION OF EXTRACELLULAR VESICLE-ASSOCIATED CD81 PROTEIN IN HUMAN GLIOMA AND NEUROBLASTOMA CELL LINES  
*Kauzlarić V, Kuharić J, Malenica Staver M, Kučić N, Sotošek Tokmadžić V, Grabušić K*
- PP16** THE EFFECT OF HYPOXIA ON SHAPE AND NUMBER OF MITOCHONDRIA  
*Lisjak D, Hribljan V, Jagečić D, Spajić S, Mitrečić D*

- PP17** THE EFFECTS OF ACUTE INTERMITTENT HYPERCAPNIA AT DIFFERENT BACKGROUND OXYGEN CONCENTRATIONS ON RENAL SYMPATHETIC NERVE ACTIVITY AND ARTERIAL BLOOD PRESSURE IN RATS  
*Madirazza K, Đogaš Z, Pavlinac Dodig I, Valić, Pecotić*
- PP18** MARKER OF ADULT UPPER CORTICAL LAYERS CUX2 IS EXPRESSED IN TRANSIENT CELL POPULATIONS OF THE HUMAN FETAL BRAIN  
*Miškić T, Krsnik Ž, Kostović I*
- PP19** EARLY DIFFERENTIATION OF HUMAN CENTRAL AMYGDALOID NUCLEUS REVEALED BY EXPRESSION OF TRANSCRIPTION FACTOR DLX6  
*Mulc D, Knezović V, Miškić T, Krsnik Ž, Kostović I, Vukšić M*
- PP20** LIPID ENVIRONMENT OF INSULIN AND LEPTIN RECEPTOR IN GLIOBLASTOMA CELLS  
*Muršić B, Viljetić B, Pap Marianna, Heffer Marija*
- PP21** OBSTRUCTION OF MESENCEPHALIC AQUEDUCT AND DEVELOPMENT OF HYDROCEPHALUS  
*Radoš M, Periša A, Živko M, Strbačko I, Jurjević I, Orešković D, Klarica M*
- PP22** NEURODEGENERATION, MICROGLYOSIS AND ASTROCYTOSIS IN THE OPTIC TRACT DURING THE FIRST WEEK FOLLOWING REPETITIVE MILD TRAUMATIC BRAIN INJURY IN WILD TYPE AND TDP-43 TRANSGENIC MICE  
*Rajić Bumber J, Pilipović K, Gržeta N, Dolenc P, Janković T, Križ J, Župan G*
- PP23** AFFERENT CONDITIONING OF MOTOR EVOKED POTENTIALS FOLLOWING TRANSCRANIAL MAGNETIC STIMULATION OF PRIMARY MOTOR CORTEX  
*Rogić Vidaković M, Jerković A, Šoda J, Vujović I, Benzon B, Đogaš Z*
- PP24** AUTOMATED ESTIMATION OF PEAK-TO-PEAK AMPLITUDE AND LATENCY OF MOTOR EVOKED POTENTIALS IN TRANSCRANIAL MAGNETIC STIMULATION STUDIES  
*Rogić Vidaković M, Jerković A, Šoda J, Vujović I, Benzon B, Đogaš Z*
- PP25** MOLECULAR CHARACTERIZATION OF INTERNEURONS IN THE HUMAN PREFRONTAL CORTEX  
*Sedmak D, Habek N, Dugandžić A, Petanjek Z*
- PP26** DENDRITIC REMODELING OF CONTROL AND TUMOR NECROSIS ALPHA DEFICIENT DENTATE GRANULE CELLS FOLLOWING ENTORHINAL CORTEX LESION IN ORGANOTYPIC TISSUE CULTURES  
*Smilovic D, Rietsche M, Deller T, Vukšić M*
- PP27** THE EXPRESSION OF NEUROPLASTIN, CALCIUM ATPASE AND SODIUM/POTASSIUM ATPASE IN THE BRAIN OF MICE LACKING TOLL-LIKE RECEPTOR 2  
*Stojanović M, Puljko B, Ilić K, Mlinac Jerković K, Radmilović M, Mitrečić D, Kalanj Bognar S*

- PP28** CHANGES OF PERINEURONAL NETS MORPHOLOGY, HYPERACTIVE BEHAVIOR AND COGNITIVE DEFICITS IN MILD PERINATAL HYPOXIC BRAIN LESION IN RATS  
*Trnski S, Ilić K, Nikolić B, Habek N, Hranilović D, Jovanov Milošević N*
- PP29** DEVELOPMENT AND CHARACTERIZATION OF A NOVEL TRANSGENIC MOUSE MODEL WITH BIOLUMINESCENT AND FLUORESCENT NEURONS FOR IN VIVO IMAGING  
*Valenta M, Gajović S, Josić P, Srakočić S*
- PP30** FATTY ACID TREATMENT AND CHOLESTEROL LOWERING DRUG SIMVASTATIN DISPLACE NEUROPLASTIN NP-65 FROM LIPID RAFTS  
*Zjalic M, Mateskovic A, Pap M, Heffer M*
- PP31** VALIDATION OF SPLIT LUCIFERASE REPORTER SYSTEM FOR THE DETECTION OF HUMAN TAU PROTEIN OLIGOMERIZATION IN LIVING YEAST CELLS  
*Zubčić K, Šimić G, Boban M*

#### 09.30 – 12.00 P2 CLINICAL NEUROSCIENCE

- PP32** ANATOMICAL SUBDIVISION OF THE SUBTHALAMIC NUCLEI  
*Almahariq F, Sedmak G, Raguz M, Chudy D*
- PP33** DEEP BRAIN STIMULATION FOR THE EARLY TREATMENT OF THE MINIMALLY CONSCIOUS STATE AND VEGETATIVE STATE  
*Chudy D*
- PP34** CORRELATION BETWEEN FUNCTIONAL CLASSIFICATION OF CHILDREN WITH CEREBRAL PALSY AND INTRACRANIAL ULTRASOUND AND MAGNETIC RESONANCE FINDINGS  
*Delin S, Šimic Klarić A, Mejaški Bošnjak V*
- PP35** VOLUMETRIC MAGNETIC RESONANCE IMAGING – BASED MORPHOMETRY AND EARLY MOTOR DEVELOPMENT OF PRETERM INFANTS  
*Katušić A, Raguz M, Žunić Išasegi, Kostović I*
- PP36** EARLY NAA AND CHO CHANGES MEASURED BY MAGNETIC RESONANCE SPECTROSCOPY IN DLPC AND AMYGDALA PREDICT LONGER DEPRESSION-FREE INTERVAL UNDER MAINTENANCE ANTIDEPRESSANT TREATMENT  
*Henigsberg N, Kalember P, Radoš M, Radoš M, Savić A, Šarac H, Šečić A, Bajš Janović M, Ozretić D, Erdeljić Turk V, Hrabač P*
- PP37** DEMONSTRATION OF NEUROFEEDBACK TRAINING IN TIC TREATMENT – A CASE STUDY  
*Kovač D, Golubić S*

**PP38** MIGRAINE HEADACHES - EFFECTS OF NEUROFEEDBACK TRAINING ON MIGRAINE WITH AURA: A CASE STUDY

*Kovač D, Golubić S*

**PP39** INFLAMMATORY PROCESSES IN DENTAL MEDICINE AND PSYCHOPHARMACOLOGICAL TREATMENT OF DEPRESSION - PRELIMINARY RESEARCH RESULTS

*Novy Radonić E, Jelavić S, Caratan S, Aurer A, Filipčić I, Radonić E*

**PP40** VOLUMETRIC INDICATORS OF RECOVERY FROM VEGETATIVE AND MINIMALLY CONSCIOUS STATE AFTER DEEP BRAIN STIMULATION

*Predrijevac N, Raguz M, Deletis V, Oreskovic, Chudy*

**PP41** DEEP BRAIN STIMULATION IS ACCOMPANIED WITH BRAIN STRUCTURE CHANGES IN VEGETATIVE AND MINIMAL CONSCIOUS STATE PATIENTS

*Raguz M, Predrijevac N, Deletis V, Almahariq F, Kostovic I, Chudy D*

### Saturday, 14th September 2019 - POSTER SESSION II

#### 14.00 – 16.30 P3 COGNITIVE NEUROSCIENCE

**PP42** PREFRONTAL CORTEX ACTIVATION DURING COGNITIVE TASK AS A PREDICTOR OF STRESS RESILIENCE: FMRI VS FNIRS STUDY

*Kesedžić I, Božek J, Radoš M, Popović S, Čosić K*

**PP43** INFLUENCE OF AGE AND GENDER ON COGNITIVE AND PSYCHOMOTOR ABILITIES MEASURED BY THE COMPLEX REACTIONMETER DRENOVAC-SERIES TESTS

*Krišto D, Pavlinac Dodig I., Lušić Kalcina L, Pecotić R, Valić M, Đogaš Z*

**PP44** POSTOPERATIVE MONITORING OF COGNITIVE FUNCTIONS AFTER LARYNGECTOMY

*Kukulj M, Tot M, Čičak K, Kopic A, Ferenčak B*

**PP45** SYSTEM FOR AUTOMATIC FEATURE EXTRACTION AND PATTERN RECOGNITION IN EEG SIGNAL ANALYSIS

*Moštak I, Friganović K, Zelenika Zeba M, Cifrek M*

**PP46** SLEEP, ANXIETY, AND COGNITIVE AND PSYCHOMOTOR ABILITIES OF MEDICAL STUDENTS MEASURED BY TESTS OF THE COMPLEX REACTIONMETER DRENOVAC SERIES

*Pavlinac D, Lušić Kalcina L, Valić M, Pecotić R, Đogaš Z*

**PP47** THE INTELLICAGE – AUTOMATED BEHAVIORAL PHENOTYPING IN RODENTS

*Russig H, Daldrup T*

**PP48** SOME DETERMINANTS OF WELL-BEING AMONG MOTHERS OF PRETERM INFANTS

*Vidaković M, Ombla, J, Nekić M*

**PP49** ROLE OF CONSERVED BIOLOGICAL PATHWAYS AND FUNCTIONING OF MISMATCH REPAIR GENES IN MENINGIOMA PROGRESSION

*Bukovac A, Kafka A, Dragičević K, Brlek P, Cesarec Augustinović S, Raguz M, Pećina-Šlaus N*

#### 14.00 – 16.30 P4 MOLECULAR NEUROSCIENCE

**PP50** NEUROPLASTIN IN THE HUMAN BRAIN

*Ilić K, Mlinac-Jerković K, Sedmak G, Šimić G, Jovanov-Milošević N, Kalanj-Bognar S*

**PP51** THE ROLE OF INNATE ELECTROMAGNETIC FORCES AROUND NEURONS - IMPLICATIONS ON MICROGLIAL AND ASTROCYTIC ACTIVITY

*Isakovic J, Holler R, Mitrecic D, Hellmich C*

**PP52** WNT SIGNALOSOME IS TARGETED IN ASTROCYTOMA

*Kafka A, Bukovac A, Njirić N, Tomas D, Brlek P, Dragičević K, Pećina-Šlaus N*

**PP53** STEM CELL POTENTIAL IN THE FETAL SUBPLATE

*Miškić T, Majić Zidarić V, Kostović I, Krsnik Ž*

**PP54** GENOMIC COPY NUMBER ABERRATIONS FOUND IN ASTROCYTOMA AND THEIR VALIDATION THROUGH CBIOPORTAL DATABASE

*Pećina-Šlaus N, Kafka A, Bukovac A, Logara M, Bakarić R, Gotovac-Jerčić K, Borovečki F*

**PP55** THE SEMAPHORIN5A DEVELOPMENTAL EXPRESSION AND FUNCTIONAL ROLE IN MICE TELEENCEPHALON

*Putar D, Zima D, Trnski S, Bobić Rasonja M, Jovanov Milošević N*

#### 14.00 – 16.30 P5 NEURODEGENERATIVE DISORDERS

**PP56** INABILITY OF TG2576 TRANSGENIC MICE TO COMPENSATE GALACTOSE-INDUCED GLUTAMATERGIC AND ENERGETIC DISRUPTION

*Babić Perhoč A, Homolak J, Knezović A, Osmanović Barilar J, Šalković-Petrišić M*

**PP57** NEUROPROTECTIVE EFFECTS OF EXERCISE IN RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE COULD BE MEDIATED BY THE ACTIVATION OF PARAVASCULAR BRAIN WASTE REMOVAL SYSTEM AND PEROXIDASE ACTIVATION

*Homolak J, Babić Perhoč A, Knezović A, Osmanović Barilar J, Šalković-Petrišić M*

**PP58** CEREBELLUM IN ALZHEIMER'S DISEASE: QUERCETIN AS A POTENTIAL THERAPEUTIC

*Kukulj M, Langer Horvat L, Šimić G, Šoštarčić B, Branović Čakanović K, Vlahović D, Gračan R, Zrinščak I, Nikolić B, Odeh D, Oršolić N*



**PP59** DEVELOPMENT OF NON-TRANSGENIC RAT TAUOPATHY MODEL BY APPLICATION OF TAU OLIGOMERS INTO THE ENTORHINAL CORTEX  
*Langer Horvat L, Španić E, Babić Leko M, Šimić G*

**PP60** SEX AND AGE SPECIFIC CENTRAL INFLAMMATION IN RATS UPON CHRONIC STRESS  
*Majer T, Bardak A, Labak I, Balog M, Gaspar R, Szűcs K, Heffer M*

**PP61** SPEECH AND LANGUAGE DISORDERS IN NEURODEGENERATIVE DISEASES – ALZHEIMER'S DEMENTIA AND PRIMARY PROGRESSIVE APHASIA – TWO CASE STUDIES  
*Nikolić Margan A*

**PP62** OXIDATION-REDUCTION CHANGES AND DISTRIBUTION OF TOXIC AND ESSENTIAL/TRACE ELEMENTS IN THE RAT BRAIN INDUCED BY ISOFLURANE AND IRON-DEXTRAN  
*Odeh D, Oršolić N, Kukolj M, Debić S, Odeh S, Bilandžić N, Sedak M*

**PP63** EXPRESSION OF THE ACTIVATED MICROGLIA CELLS MARKERS IN ALZHEIMER'S DISEASE  
*Španić E, Babić Leko M, Ilić K, Langer Horvat L, Šimić G*

#### **14.00 – 16.30 P6 NEURODEVELOPMENTAL BASIS OF COGNITIVE, MENTAL AND NEUROLOGICAL DISORDERS**

**PP64** EVIDENCE FOR DECREASED DENSITY OF CALRETININ-IMMUNOPOSITIVE NEURONS IN THE CAUDATE NUCLEUS IN PATIENTS WITH SCHIZOPHRENIA  
*Adorjan I, Bin S, Feher V, Tyler T, Veres D, Chance SA, Szele FG*

**PP65** ANALYSIS OF THE MEF2C TRANSCRIPTION FACTOR EXPRESSION IN DEVELOPING HUMAN CINGULATE GYRUS  
*Bobić-Rasonja M, Štajduhar A, Sedmak G, Petrović D, Jovanov-Milošević N*

**PP66** PREFONTAL CORTEX IN NEURODEVELOPMENTAL DISORDERS: INSIGHTS FROM THE ORGANIZATION OF BRODMANN AREA 10 IN WILLIAMS SYNDROME  
*Hrvoy Mihic B, Hanson K, Lew C, August I, Cuevas D, Greiner D, Jovanov Milosevic N, Petanjek Z, Semendeferi K*

**PP67** TESTING PRESENCE OF DYSKINETIC EYE MOVEMENT DISORDER IN CHILDREN WITH DYSKINETIC CEREBRAL PALSY  
*Ivošević M, Alimović S, Moslavac A, Bošnjak Nađ K, Mejaški-Bošnjak V*

#### **14.00 – 16.30 P7 NEUROPHARMACOLOGY**

**PP68** ETHANOLIC EXTRACT OF PROPOLIS EXACERBATES COPPER-INDUCED NEURONAL DEATH: THE INVOLVEMENT OF ROS/P53/P38 INTERACTIONS  
*Ivošević M, Alimović S, Moslavac A, Bošnjak Nađ K, Mejaški-Bošnjak V*

#### **14.00 – 16.30 P8 SLEEP**

**PP69** INTRODUCING THE SLOPE OF THE OXYGEN DESATURATION CURVE AS A NOVEL INDEX IN ASSESSING PHENOTYPES IN SEVERE OSA PATIENTS  
*Demirović S, Lušić Kalcina L, Pavlinac Dodig I, Pecotić R, Valić M, Đogaš Z*

**PP70** POLYSOMNOGRAPHY PARAMETERS AND SLEEP ARCHITECTURE: THE ROLE IN DAYTIME SLEEPINESS AND SLEEP QUALITY OF OSA PATIENTS  
*Lušić Kalcina L, Pavlinac Dodig I, Pecotić R, Valić M, Đogaš Z*

**PP71** SHORT LATENCY AFFERENT CORTICAL INHIBITION IN OBSTRUCTIVE SLEEP APNEA SYNDROME: A TMS STUDY  
*Rogić Vidaković M, Jerković A, Šoda J, Vujović I, Benzon B, Đogaš Z*

**PP72** SLEEP ARCHITECTURE IN SEVERE OSA PATIENTS  
*Valić M, Valić Z, Pecotić R, Lusić Kalcina L, Pavlinac Dodig I, Đogaš Z*

#### **14.00 – 16.30 P9 HYPOXIC-ISCHEMIC DAMAGE**

**PP73** COMBINING IN VIVO IMAGING MODALITIES IN EVALUATION OF MOUSE BRAIN AFTER ISCHEMIC LESION  
*Gajović S, Škokić S, Gorup D, Dobrivojević Radmilović M, Glasnović A, Josić P, Srakočić S*

**PP74** EFFECTS OF NEONATAL NORMOBARIC HYPOXIA ON RAT BEHAVIOR AT YOUNG AND ADULT AGE  
*Nikolić B, Trnski S, Kološnjaj I, Stipan M, Hranilović D, Jovanov Milošević N*

**PP75** THE ROLE OF GUANYLATE CYCLASE-C ON ISCHEMIC STROKE  
*Ratko M, N. Habak N, Dobrivojević Radmilović M, Škokić S, Justić H, Dugandžić A*

**PP76** MATURATIONAL CHANGES OF WHITE MATTER SEGMENTS IN PRETERM CHILDREN REVEALED BY FA AND ADC VALUES  
*Žunić Išasegi I, Štajduhar A, Raguž M, Radoš M, Kostović I*

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# A B S T R A C T S

## BUILDING THE HUMAN BRAIN: MOLECULAR LOGIC OF NEURAL CIRCUIT FORMATION

Nenad Sestan

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The question of what makes us human has fascinated humankind throughout modern history. Today, we view the brain as the core component of human identity, and an understanding of this organ is consequently essential for answering why we as a species are what we are. What distinguishes humans from other species is largely thought to reside in the unique features of brain development, especially in the wiring of the immensely complex neural circuits that underlie our remarkable cognitive and motor abilities. However, the unique innovations driving the formation of these intricate neural circuits may also increase our susceptibility to certain neurological and psychiatric disorders.

In my presentation, I will describe some of our recent efforts to understand the molecular mechanisms by which the connections between neurons are formed in the cerebral cortex, the part of the brain that processes senses, commands motor activity, and underlies higher-order cognitive functions. I will also present evidence on how this complex developmental process may have evolved and become compromised in human disorders.

## THE DIVERSITY OF CAJAL-RETZIUS NEURONS IN THE DEVELOPING HUMAN CORTEX

Gundela Meyer

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Cajal-Retzius neurons (CRN) are the dominant cell population in the fetal marginal zone, the future layer I, and the main source of Reelin, a secreted glycoprotein essential for laminar positioning of radially migrating neurons destined for the cortical plate. Human CRN represent a heterogeneous cell population with regard to their origins, time of birth, morphological differentiation, and fate, but they have in common the co-expression of Reelin with transcription factors Tbr1 and p73. Near midgestation CRN attain uniquely complex morphologies and extend a prominent axonal plexus which breaks down around the 25th postconceptional week, at the onset of cortical folding. In addition, human CRN express a large variety of transcripts and proteins which points to interactions with adjacent structures (basement membrane, radial glia endfeet, meninges and associated blood vessels) and activities related to important signalling pathways (e.g. retinoic acid, nitric oxide, CXCL12/CXCR4). In human cortex, CRN function might thus be considered in a wider context, possibly independent from Reelin/Dab1 signalling. In the developing hippocampus, expression of p73 in CRN is required for the formation of the hippocampal fissure. This raises the question of whether CRN may be involved in cortical gyration. The diversity and developmental dynamics of CRN were thus examined in a different model of a large and highly gyrated cortex, in the fetal wild boar (*Sus scrofa*). In wild boar embryos, CRN appear early and seem to belong to a single population displaying a rather simple, rodent-like morphology. The complex morphotypes of human CRN, and their sequential appearances in the marginal zone are not present in the fetal boar, and may represent a human or primate-specific acquisition.

## CEREBRAL CORTEX DYSGENESES: GENE REGULATION, MOLECULAR PATHWAYS AND CNS DISEASE

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Brain malformations are an important cause of neurological disease in infants and children. Malformations are usually the result of abnormal neuronal or glial proliferation or migration and can be divided into several groups<sup>1</sup>: (1) diffuse neuronal or glial *dysgenesis* (leading to a dysmorphic brain), (2) cerebral *hypogenesis* (leading to microcephaly), (3) localized *dysgenesis* due to proliferation of *abnormal cell types* (non-neoplastic), (4) *dysgenesis* due to *abnormal cell migration* (abnormal early migration, abnormal late migration or overmigration through the pial limiting membrane), and (5) malformations due to *abnormal postmigrational development*. Group 1, diffuse cerebral dysgeneses, result from the production of abnormal (but non-neoplastic) cell types; these patients usually manifest abnormal neurodevelopment and epilepsy, as the result of disorders such as focal cortical dysplasias, Tuberous Sclerosis (with "cortical hamartomas" that are identical to focal cortical dysplasias and are the result of late or very localized mutations), and hemimegalencephalies (which are due to very early or large areas of mutation<sup>2</sup>. Group 2, cerebral hypogenesis due to decreased proliferation, results in microcephaly, of which there are many types<sup>3,4</sup>; our understanding of these disorders has increased substantially in recent years with the discovery of the genes responsible and their protein products; most of the responsible genes function in cell replication, but the phenotype varies widely as a result of the specific gene and specific mutation. Group 3, localized dysgenesis due to proliferation of abnormal cell types, largely results from mutations of genes in the mTOR pathway<sup>2,3,5</sup>. Malformations secondary to mutations of Tubulins (particularly TUBA1A) and microtubule-associated proteins also fit into this group of disorders<sup>6</sup>, with affected patients showing microcephaly, altered gyration of the cerebral cortex, small/absent corpus callosum and cerebellar anomalies. Of note, *although TUBA1A children consistently have multiple brain anomalies, those anomalies vary considerably, and Genotype-Phenotype correlation is unable to be established*<sup>6</sup>. This is true in many diseases, and suggests that *genotype directs initial phenotype but environmental effects are at least as important*. Groups 4 and 5 consist of patients with abnormal neuron migration, which can occur during early phases of neuronal migration, when the immature neurons still move among radial glia, to later phases when they depend upon proper attachment of distal radial glia to the glia limitans; abnormal distal attachment may result in development of "gaps" in the glia limitans with consequent overmigration of neurons into the subarachnoid space, causing a thick, irregular (sometimes called "cobblestone" cortex)<sup>7-9</sup>. Other "variant lissencephalies" have also been described, caused by variants of mutations in the ARX and Reelin (and other) signaling pathways, with the precise variation depending upon the gene(s) affected and the severity of the effect of the altered chemical bonding upon the function of the pathway.

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## POST-TRANSCRIPTIONAL EVENTS IN DEVELOPING NEOCORTEX

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Neocortical development requires precise spatiotemporal regulation to generate a properly functioning central nervous system. The distinct neocortical developmental stages are regulated by changes in gene expression (transcription) and protein synthesis (translation). Time-dependent gene transcription is thought to be a major regulator central nervous system development overall, but time-dependent regulation of mRNA translation is also emerging as a key control mechanism. Indeed, we found that RNA binding proteins (RBP) control spatiotemporal mRNA translation events during neocortical development and dictate differentiation of distinct neuronal subtypes. Interestingly, roles of these RBPs are under the control of timed secreted factors delivered by ingrowing thalamo-cortical axons. Discoveries of these novel molecular and cellular mechanisms during neocortical development may open avenues for better understandings of neurodevelopmental disorders associated with abnormal NSCs, mRNA translation, and/or central nervous system development and regeneration.

## QUANTITATIVE IN VIVO MRI ASSESSMENT OF STRUCTURAL ASYMMETRIES AND SEXUAL DIMORPHISM OF TRANSIENT FETAL COMPARTMENTS IN THE HUMAN CEREBRAL CORTEX

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Structural asymmetries and sexual dimorphism of the human cerebral cortex have been identified in newborns, infants, children, adolescents, and adults. Some of these findings were linked with cognitive and neuropsychiatric disorders, which have roots in altered prenatal brain development. Nonetheless, besides the structural asymmetries in the curvature of the cerebral cortex, little is known about structural asymmetries or sexual dimorphism of transient fetal compartments that occur during the prenatal brain development. In this study, we have used in-vivo structural T2-weighted MRIs of 42 healthy fetuses (16.43-36.86 gestational weeks (GW), 15 females) to identify structural asymmetries and sexual dimorphism of volumes of 22 manually delineated regions of transient fetal compartments (cortical plate and subplate). We found significant leftward asymmetry in the volume of the cortical plate, and the volume of subplate in inferior frontal gyrus. Orbitofrontal cortex showed significant rightward asymmetry in the volume of cortical plate merged with subplate. Significantly larger volumes were found in males in regions belonging to limbic (cingulate gyrus), occipital (pericalcarine cortex), and frontal (inferior frontal gyrus) lobes. However, in these regions, the sexual dimorphism in volume was driven by the significantly larger volume of the subplate compartment. Lastly, we did not observe hemispheric asymmetries or sexual dimorphism in the growth trajectories of the cortical plate or subplate. In conclusion, these results support a hypothesis that structural asymmetries and sexual dimorphism in relative volumes of cortical regions are present during prenatal brain development but are not age dependent.

## NEURONAL CHOLESTEROL BIOSYNTHESIS AND HOMEOSTASIS

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Although the human brain only accounts for about 2% of total body weight, it contains as much as 25% of the body's cholesterol and cholesterol derivatives. The metabolism of brain cholesterol differs markedly from that of other tissues, and it fully relies on *de novo* synthesis in the nervous tissue, as the blood-brain barrier prevents the uptake of cholesterol from the circulation. Sterol homeostasis disturbances are critical components of many brain disorders. Dysfunction of the cholesterol biosynthesis pathways and/or metabolism leads or contributes to a number of neurodevelopmental and neurodegenerative disorders such as Smith-Lemli-Opitz Syndrome (SLOS), Niemann-Pick type C (NPC) disease, desmosterolosis, Huntington's disease, and Alzheimer's disease. Intact cholesterol metabolism is also essential for normal function of the adult brain: in the elderly, high cholesterol is associated with better memory function, while low cholesterol is associated with an increased risk for depression.

The function of cholesterol in the CNS extends beyond being a structural component of cellular membranes and lipid rafts: it is required for synapse and dendrite formation, axonal guidance, and serves as a precursor for various biosynthetic pathways. Cholesterol biosynthesis occurs by the same mechanism across all tissues, yet cholesterol metabolism is tissue-specific. Across all tissues, two parallel and interlinked enzyme cascades, named Kandutsch-Russell (KR) and Bloch pathways, perform sterol biosynthesis. In the brain, it has been proposed that neurons preferentially use the KR pathway, while glia are relying on the Bloch pathway, but there has been limited amount of experimental evidence to support this claim.

It is widely believed that the main sterol synthesis in the adult brain is performed by glial cells. However, our studies show that neurons express genes encoding cholesterol biosynthesis enzymes, and it appears that during the neuronal membrane growth (and maximal cholesterol demand), neuronal cholesterol biosynthesis is indispensable. Our most recent studies revealed that developing neurons synthesize their endogenous cholesterol independently from astrocytic sterol synthesis and that both developing neurons and astrocytes release cholesterol into their local environment. In addition, steady-state levels of cholesterol are higher in neurons than in astrocytes, with significantly higher amount of cholesterol produced in neurons. Neuronal sterol synthesis increases over time, while astrocytes maintain a strict control of their endogenous cholesterol homeostasis. Our study also unequivocally shows that developing neurons have both an active cholesterol biosynthesis and an ApoE-dependent uptake. In contrast, astrocytes can utilize free sterols from the extracellular milieu. Finally, both developing neurons and astroglia preferentially use the Bloch sterol biosynthesis pathway, where desmosterol is the immediate precursor to cholesterol, and that the contribution of the KR pathway to endogenous biosynthesis is negligible in the brain tissue.

## CHARACTERIZING NEURO-BEHAVIORAL GEOMETRY EMBEDDING VIA NEUROPHARMACOLOGY, TRANSCRIPTOMICS AND CLINICAL NEUROIMAGING

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A key challenge in the field of neuropsychiatry lies in matching patients with effective treatments. Most studies in psychiatry operate under the canonical assumption that categorical diagnostic clinical grouping and/or pre-existing clinical assessments are the 'gold standard' for describing behavioral - and therefore neural - variation in patients. Attempts to robustly characterize the neural substrates of these predefined variables have yielded limited success, suggesting an inadequate mapping to neurobiologically meaningful variation. Notably, a great deal of heterogeneity exists even within groups of patients with the same categorical diagnosis. Thus, understanding the mapping between specific behaviors and clinically-meaningful variation in neural properties is critical to develop and ultimately administer effective individualized neurobehavioral treatments.

Here, we describe a multivariate neuro-behavioral framework under which behavioral variation can be mapped to features of specific neural systems in a data-driven way. We leverage neural (fMRI-derived) and behavioral data from large-scale datasets across the psychosis spectrum that were publicly available via the NIMH Data Archive as part of the Bipolar & Schizophrenia Consortium for Parsing Intermediate Phenotypes study. We relate these findings to effects from two pharmacological neuroimaging experiments manipulating the NMDA glutamate receptor via ketamine and the 5-HT receptor via LSD. In turn, we link the identified maps to patterns of brain-wide transcriptomic variation derived from the Allen Human Brain Atlas (AHBA). We first identify dimensions of maximal behavioral variation in patients by performing dimensionality reduction analysis across all behavioral measures. Importantly, these dimensions are not parallel to traditional clinical symptom scales derived from pre-existing clinical instruments used in psychiatry, and do not reflect conventional categorical diagnostic boundaries. We then demonstrate that variation along identified behavioral dimensions relates to variation in specific neural systems, using a data-driven measure of functional connectivity. Critically, these neuro-behavioral relationships were not observed using either traditional diagnostic groups or a priori clinical scales. We further show that this framework can inform the identification of pharmacological neural circuit targets for specific symptom profiles at the individual subject-level. Characterizing how and which specific sets of symptoms map to neural circuitry is a key step towards developing targeted and effective treatments for psychiatric disorders. We propose the Neuro-Behavioral Relationships In Dimensional Geometric Embedding (N-BRIDGE) framework as a key step towards unified mapping between the geometry of data-driven neuro-behavioral variation. In summary, we detail a general framework for linking neuro-behavioral variation along the psychosis spectrum with neuropharmacological effects and genetic findings that can point to personalized treatment-relevant mechanism.

## GWAS-IDENTIFIED ALZHEIMER'S DISEASE RISK VARIANTS - LESSONS FROM NEUROPATHOLOGY

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The elucidation of the pathophysiologic mechanisms of late onset Alzheimer's disease (AD) has lately been driven by genome-wide association studies (GWAS) to identify specific genes or genomic regions that are associated with disease risk. However, the information on the expression pattern of these genes' protein products in human brain, in health as well as in disease, is almost universally lacking. Our studies closed that gap of knowledge for two genes: 1) BIN1 (Bridging integrator 1) which harbors the second most common AD risk variant after APOE4, and 2) MSRB3 (Methionine Sulfoxide Reductase-B3) containing SNP rs61921502 associated with both AD and low hippocampal volume. In quantitative analyses of BIN1 signal in CA1 region during AD progression we found: 1) sustained expression in glial cells, 2) decreased area of neuropil expression and 3) increased cytoplasmic neuronal expression that did not correlate with neurofibrillary tangle load. In AD patients, both prefrontal cortex and CA1 region showed significant decrease in BIN1-immunoreactive neuropil area and significant increase in the number of BIN1-positive neurons. The number of CA1 BIN1-immunoreactive pyramidal neurons correlated highly with hippocampal CERAD neuritic plaque score while BIN1 neuropil signal was absent at neuritic plaque sites. Studies to elucidate how BIN1 risk variants affect the myelin-axon unit, amyloid and tau accumulation, and glial homeostasis are underway.

To begin exploring the potential role of MSRB3 protein in the human hippocampal pathology associated with cognitive decline in AD, we investigated MSRB3-immunoreactivity in postmortem human hippocampi, accompanied with neuropathologic reports and clinical dementia rating (CDR) scores. To uncover cellular organelles that associated with MSRB3 signal we performed ultrastructural analysis in rodent hippocampi, which mimicked human MSRB3 expression, to find MSRB3 signal in synaptic vesicles in CA3 and CA1. To evaluate the relationship between the sites of expression of MSRB3 and of synaptic vesicles-associated proteins, we performed immunohistofluorescence to find co-localization of MSRB3 with vesicle associated membrane protein 2 (VAMP2) and vesicular glutamate transporter 1 (VGLUT1). The discovered MSRB3 signal in the hippocampal white matter arteriolar walls, decreased in AD, prompted us to investigate the relationship between the MSRB3 SNP rs61921502, G (minor/risk allele), and MRI measures of brain injury (total brain volume, hippocampal volume, and white matter hyperintensities), brain infarcts, and the incidence of stroke, dementia, and AD in 2,038 Framingham Heart Study Offspring participants with MRI administered close to examination cycle 7 (1998-2001). We found MSRB3 rs61921502 minor/risk allele to be significantly associated with increased odds for MRI brain infarcts in the absence of APOE4. These results suggest multifaceted interplay between APOE4 and MSRB3 rs61921502 minor/risk allele, influencing the decline of hippocampal vascular health and cognitive functions.

## MOLECULAR BASES OF CHOLESTEROL BIOSYNTHESIS DISORDERS

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Cholesterol is an essential precursor for various biologically important molecules including hormones, vitamin D and bile acids. It also plays an integral role in maintaining cell membrane structure and function. Cholesterol's role is even more important in the brain, where roughly 25% of the body's total cholesterol resides despite the fact that it accounts for only 2% of total body weight. Cholesterol biosynthesis is a complex, multi-step process involving over thirty enzymes. Mutations in sterol enzymes lead to several neurodevelopmental disorders. Two examples include Smith-Lemli-Opitz Syndrome (SLOS) and Desmosterolosis (Desm). SLOS is an autosomal recessive disorder caused by mutations in DHCR7, and SLOS transgenic mouse models recapitulate molecular and biochemical changes seen in SLOS patients. Similarly, DHCR24 mutations in desmosterolosis patients share the same fundamental phenotype with the Dhcr24 knockout mice: they have highly elevated desmosterol, and diminished levels of cholesterol.

While desmosterol is greatly elevated in Dhcr24-KO and significantly decreased in Dhcr7-KO mice, 7DHC is increased in both of these two mouse models, and both disorders are characterized by markedly decreased cholesterol levels in the brain. Thus, it is perhaps not surprising that the molecular and microanatomical changes also show some similarities between Dhcr24-KO and Dhcr7-KO mice. Namely, the affected gene expression, while not identical across the two mouse models, impact the same transcriptional networks, and increased neuronal arborization is a feature of both models. Untangling the precise origin of all these changes is challenging and we propose that the primary driver of the majority of common changes is lack of cholesterol, while the gene-KO specific changes are due to elevated 7DHC or desmosterol levels. 7DHC is the most oxidizable lipid molecule known to date leading to over a dozen oxidation products, 7DHC-derived oxysterols. Several novel 7DHC-derived oxysterols have been identified. The build-up of these oxysterols leads to gene expression changes, affects neuronal signaling and leads to morphological changes. As such, the increase in these intermediates that are readily oxidizable likely contributes to the pathogenesis of SLOS and DESM.



## NEUROPHYSIOLOGY OF THE EXTRATERRESTRIAL SLEEP: POSSIBILITY OR CULPABILITY?

Ivana Rosenzweig

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The effects of microgravity during spaceflight or prolonged bed-rest on the brain have received particular attention relating to the intracranial pressure syndrome in astronauts returning from the International Space Station. There have, however, been no extensive studies of associated changes in neuroanatomy during wake and sleep. Significantly disrupted mood and cognition has also been consistently reported in space missions. Recent plans for manned missions to Mars and other planets have highlighted our ignorance of these and related problems, and they stress the importance of urgent human studies.

The aim of the multimodal-imaging study which will be presented during this talk, was to determine the effects of seven days of supine unloading on a supersaturated saline-filled water bed (hyper-buoyancy floatation, HBF), a novel Earth-based microgravity analogue. We explored a variety of cognitive and neurophysiological changes during various levels of consciousness during conditions of microgravity and sensory deprivation, with particular emphasis to sleep changes. This project was part of an ongoing complex multimodal-imaging study that has involved further neurophysiology and neuroimaging investigations.

## BUILDING ON 50 YEARS OF EXPERIENCE TO SHAPE MY RESEARCH TODAY (AND IN THE FUTURE)

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All my professional life I worked on the origin of human-specific cortical distinctions. Although the basic principles of cellular organization and development of the cerebral cortex in all mammals are deceptively similar, modifications of the early developmental events during myriad years of evolution have produced not only quantitative, but also qualitative changes with large functional consequences. I was investigating how these differences emerge during individual development and evolution. My initial research led to the "radial unit" and related "protomap" hypotheses which are used to explain how the complex, three-dimensional, laminar and modular structure of the large cerebral cortex is built from an initially simple two-dimensional layer of neural stem cells in the proliferative zones near the cerebral ventricles. As methods become more sophisticated, we used various in vitro and in vivo assays in the developing mouse, macaque monkey and human cerebrum to identify genes, non-coding DNA regulatory elements (promoters and enhancers) and signaling molecules engaged in the initial production of neurons from the radial glia and their fate determination, followed by migration and synaptogenesis. We found considerable species-specific differences, including the life-long permanency of neurons that is essential for the function of the human cerebral cortex as the organ of thought. This multidisciplinary research revealed that various genetic and environmental factors can induce not only gross, but also slight, inappropriate neuronal positioning—not detectable by histological examination and MRI scans—that contributes to a variety of idiopathic developmental disorders and intellectual disabilities. The talk will conclude with how this approach can inspire strategies for prevention and therapy of congenital neuropsychiatric disorders.

## CORTICAL LAYER WITH NO KNOWN FUNCTION

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The lowermost cell layer of the cerebral cortex that contains interstitial white matter cells in humans has great clinical relevance (Kostovic and Rakic, 1990; Kostovic et al., 2019). These neurons express higher proportions of susceptibility genes linked to human cognitive disorders than any other cortical layer and their distribution is known to be altered in schizophrenia and autism (Hoerder-Suabedissen et al., 2013; Bakken et al., 2016). In spite of these clinical links, our current knowledge on the adult layer 6b is limited. These cells are the remnants of the subplate cells that are present in large numbers (for example see Swiegers et al., 2019) and play key role in the formation of cortical circuits but a large fraction of them die during postnatal development. The adult population that remains in all mammals to form interstitial white matter cells in human or layer 6b in mouse display unique conserved gene expression and connectivity (Hoerder-Suabedissen et al., 2018; Boon et al., 2019). Subplate neurons also have secretory functions during cortical development (Kondo et al., 2015; Adorján et al., 2019). We study their input and output using combined anatomical, genetic and physiological approaches in the mouse. Selected cortical areas, relevant for sensory perception, arousal and sleep (V1, S1, M1, prefrontal cortex) are studied using chemogenetic and optogenetic methods. Our preliminary data suggest that 6b is not just a developmental remnant cell population in the adult, but a layer that plays a key role in cortical state control, integrating and modulating information processing (Guidi et al., 2016).

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# POSTER PRESENTATIONS

## PP 1

**RAPID GOLGI IMPREGNATION OF PYRAMIDAL NEURONS IN THE PREFRONTAL CORTEX IN SCHIZOPHRENIA**Banovac I<sup>1,2,3</sup>, Sedmak D<sup>1,2,3</sup>, Rojnić Kuzman M<sup>4</sup>, Hladnik A<sup>1,2,3</sup>, Petanjek Z<sup>1,2,3</sup>

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Abnormalities in oligodendrocytes lead to altered myelination in schizophrenia, according to most recent studies. The degree of myelination affects axon impregnation in Golgi staining. Therefore, the aim of this study is to compare the axon impregnation on rapid Golgi between schizophrenic and control subjects.

We analyzed sections of the prefrontal cortex containing Brodmann area 9 in five schizophrenic and five control subjects. The sections were stained using the rapid Golgi method and the axons of randomly selected pyramidal neurons of layer III and V were reconstructed using Neurolucida 4 software. The axon impregnation lengths were then compared between the schizophrenic and control groups.

Our results showed an increase in the length of axonal staining of the pyramidal neurons in the prefrontal cortices of schizophrenic subjects. The length of the stained axon main trunk was  $132.5 \pm 63.5 \mu\text{m}$  in the schizophrenic group and  $64.8 \pm 20.2 \mu\text{m}$  in the control group. The difference was shown to be statistically significant (p-value on Student's t-test was  $<0.05$ ). Our analysis also revealed that more axon collaterals per neuron were stained in the schizophrenic group (14.5% on average) than in the control group (5.1% on average).

The increased axonal staining in schizophrenic subjects could be explained by reduced myelination of the axons, which allows for better impregnation on Golgi staining. Such a decrease in axon myelination is in line with the neurodevelopmental model of schizophrenia, which proposes that pathology in schizophrenia is more dependent on secondary effects, rather than on a primary lesion. In conclusion, our results support that the cortical circuitry disorganization in schizophrenia is caused by oligodendrocyte abnormalities which lead to a decrease in axon myelination.

## PP2

**MORPHOLOGICAL ANALYSIS OF VON ECONOMO NEURONS**Banovac I<sup>1,2,3</sup>, Sedmak D<sup>1,2,3</sup>, Džaja D<sup>1,2,3</sup>, Jalšovec D<sup>1</sup>, Jovanov Milošević N<sup>2,3,4</sup>, Rašin MR<sup>5</sup>, Petanjek Z<sup>1,2,3</sup>

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Von Economo neurons (VENs) are modified pyramidal neurons abundant in layer V of the anterior cingulate cortex (ACC) and fronto-insular cortex (FI) of the human brain. The aim of this study is to establish a comprehensive morphological description of VENs in the human ACC.

We analyzed sections of the ACC (Brodmann area 24) in five adult human specimens. The sections were stained using rapid Golgi, Golgi-Cox and Nissl.

VENs have a distinct somato-dendritic morphology that allows their clear distinction from other modified pyramidal neurons. We established that VENs have a perpendicularly oriented, rod-like core part consisting of the cell body and two thick extensions – the apical and basal stem. The core part was characterized by a lack of a clear demarcation between the cell body and the two extensions. Another typical feature was the basal extension which ended in a brush-like branching pattern. The most distinct feature of VENs was the distant origin site of the axon, which arose from the ending of the basal extension. Quantitative analysis found that VENs could be divided into two groups based on total dendritic length – small VENs with a peak total dendritic length of 1500 – 2500  $\mu\text{m}$  and large VENs with a peak total dendritic length of 5000 – 6000  $\mu\text{m}$ . Comparative morphological analysis of VENs and other oval and fusiform modified pyramidal neurons showed that on Nissl sections oval and fusiform neurons could be misidentified as VENs.

Our data show that the reports on the presence of VENs in non-primates in other layers and regions of the cortex need further confirmation by showing the dendritic and axonal morphology of these cells. In conclusion, our study provides a foundation for further comprehensive morphological and functional studies on VENs between different species.

## PP3

### SMARTPHONE OPHTHALMOSCOPY – A NOVEL METHOD FOR TRACKING CHANGES IN THE RETINA AFTER ISCHEMIC STROKE IN DIABETIC MICE

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Retinal damage is a common occurrence after ischemic stroke. The retinal circulation could be used as a potential diagnostic fingerprint of cerebral vascular damage since they share similar origin and drainage, which can be easily visualized using ophthalmoscopy. The aim of the current study was to establish a novel method for assessment of retinal vascular changes after ischemic stroke in diabetic mice.

C57Bl6 albino and C57Bl6 mice were fed a high fat diet for 12 weeks to induce type II diabetes which was confirmed and later monitored by fasting blood glucose and HbA1c levels. After successful diabetes induction mice underwent a transient middle cerebral artery occlusion (MCAO) followed by reperfusion. Seven days prior to MCAO and 2, 7 and 35 days after surgery the animals were scored for neurological deficit and imaged by a 7T BioSpec MRI system. We introduced a novel smartphone fundus photography and fluorescein angiography method to monitor longitudinally the changes in the retinal vasculature after MCAO. Our system works as an ophthalmoscope capturing a digital image of the fundus in the smartphone camera. For fluorescein angiography an excitation filter was placed in front of the light source and a barrier filter in front of the camera lens. The obtained images were analyzed using an automated software developed in our laboratory. Type II diabetes was successfully induced and maintained for the whole duration of the study. Using MRI, we were able to non-invasively monitor the evolution, progression and resolution of the ischemic brain lesion up to 35 days after surgery. Our novel smartphone ophthalmoscope allowed us to assess the retinal vasculature in vivo and quantify different indicators of the retinal health status. The newly established methodology allows longitudinal in vivo assessment of retinal vascular changes after ischemic stroke in diabetic mice.

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## PP4

### STRIATAL MEDIUM SPINY NEURONS SHOW GENDER DIFFERENCES IN DENDRITIC MORPHOLOGY

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The caudato-putamen complex (striatum) in rodents is the site of convergence of a wide range of cortical and subcortical inputs so the dysfunction of any of those pathways can be expressed in various changes in behavior of animals. The most numerous population of striatal neurons are medium spiny projection neurons (MSNs). For better understanding of the striatal circuitry structure and function it is important to gather data about the morphology of the MSNs especially in the context of gender differences. It is known that MSNs express membrane estrogen receptor and exhibit gender differences in the electrophysiological studies. Here we present a detailed morphological analysis of MSNs in the mice dorsal striatum for which purpose we have used brains of 15 adult C57BL/6 wild type mice (8 males and 7 females). The brain tissue was sliced in 10 mm thick blocks and stained with the FD Rapid GolgiStain TM kit. For the 3D dendritic tree reconstruction of total 162 MSNs we used a motorized microscope-computer based system and NeuroLucida software version 10.

The measured dendritic parameters have shown female animals to have statistically larger dendritic trees compared to males. Interestingly, although not statistically significant only the number of dendritic spines has shown an opposite tendency.

This finding corresponds to the previous physiological studies, and suggests that electrophysiological differences between males and females are significantly affected by differences in dendritic morphology of MSNs between them.

## PP5

**GROWTH OF HUMAN STRIATUM DURING FETAL AND EARLY POSTNATAL LIFE REVEALED BY VOLUMETRIC ANALYSIS**

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Development of striatum begins around 7th PCW when first cells originating in ganglionic eminence migrate and form striatal anlage. First inhomogeneities in internal organization are visible between 10th and 14th PCW. Development of internal organization has its peak between 20th and 24th PCW. After this period striatum undergoes perinatal and postnatal reorganization. The exact time of adult type of organization is not precisely determined in literature. The purpose of this study was to determine growth curve of striatum in order to correlate it with changes in intrinsic organization which underway in our laboratory. In addition, we correlate changes in volume with growth of hemispheric volume during fetal and early postnatal period, based on well-known corticostriatal relationship.

Study involved 5 "normal" term infants, 15 normotypic developing premature infants with normal MRI and clinical parameters, 15 postmortem fetal brains from 13 till 40 PCW (Zagreb Neuroembryological Collection) and 5 normal infants age 1 month till 1 year. All groups were scanned by MRI device, control group at term, normotypic at the corrected term age and fetal brains after insertion in formalin. All volumes were measured on 3T MR images utilizing semi-automated and manual segmentation methods (MNI toolbox, available on the Internet). All data were analyzed using MathCalc statistical software (v 4.1, USA). Linear growth was observed in all measured data. Linear growth of striatum components, both caudate and putamen, left or right, follows increased volume of hemispheres and total brain volume from 13 PCW till age of one year postnatal. Peak of volume is around 24th PCW with the fastest growth between 20 and 28 PCW.

Our study showed linear growth of observed structures (volume of nucleus caudatus, putamen and hemisphere) in fetal, neonatal and postnatal period with the peak of volume around 24th PCW. Showed results with peak around 24th PCW and the fastest growth between 20th and 28th PCW respond to transition according to structure of adult brain with modular organization which rearranges until the end of postnatal period (1st year of life). It is interesting that peak of the volume is achieved during maximum expression of modular organization which also reflex intensive ingrowth of corticostriatal projections. The normative data we presented in this study will be used for correlation with development of modular organization and analysis of brains after hypoxic lesions.

## PP6

**DO SUBTHALAMIC AND SUBSTANTIA NIGRA NEURONS SHARE COMMON NEURONAL LINEAGE?**Bokulić E<sup>1</sup>, Medenica T<sup>1,2</sup>, Sedmak G<sup>1</sup>.<sup>1</sup>*Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia,*<sup>2</sup>*Faculty of Science, Department of Biology, University of Zagreb, Croatia*

The subthalamic nucleus (STN) is a small, biconvex nucleus in the diencephalon, lying rostrally from the internal capsule to substantia nigra caudally. To this day, two theories have been postulated about the developmental origin of the STN. One theory proposes that the STN originates from the separate longitudinal subthalamic zone between the ventral thalamus and the hypothalamus, while the other suggests that the nucleus originates from the germinative zone lying caudally from the mammillary recess. Novel studies investigating the development of mesencephalic dopaminergic (mesDA) neurons suggested, based on the expression profile of several transcriptional factors, that STN and mesDA neurons may have common neuronal lineage. However, the results of these studies are inconsistent and sometimes conflicting. Furthermore, majority of published studies were conducted using mouse brain, therefore leaving the question of possible interspecies differences unanswered. To further explore these developmental theories, we employed immunohistochemical staining to study the expression of several transcription factors (Foxp1, Foxp2, Foxa1, Barhl1, Dbx1, Nkx2.1) in the STN and SN of adult mouse, rat, and human. Data from our preliminary study indicate that some STN and SN neuronal populations have a common neuronal lineage, although some of the aforementioned transcription factors are expressed exclusively in the STN. These transcription factors could help us unravel the mystery of the STN development.

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## PP7

**SYNAPTIC CHANGES WITHOUT SIGNS OF NEURODEGENERATION IN THE ROSTRAL CEREBRUM AFTER TRAUMATIC BRAIN INJURY IN THE RAT**Dolenec P<sup>1</sup>, Pilipović K<sup>1</sup>, Gržeta N<sup>1</sup>, Župan Ž<sup>2,3</sup>, Župan G<sup>1</sup><sup>1</sup>Department of Pharmacology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia,<sup>2</sup>Department of Anesthesiology, Reanimatology and Intensive Care Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, <sup>3</sup>Clinics of Anesthesiology and Intensive Care Medicine, Clinical Hospital Center Rijeka, Rijeka, Croatia

Traumatic brain injury (TBI) results from the primary damage of the brain tissue, followed by secondary events which can develop within days and months after the impact and usually contribute to further impairment. Most of the studies so far were focused on the TBI consequences at and near the injury site, even though there are many indications that damage could be widespread throughout the brain. Processes of synaptic damage, reorganization and repair following TBI could be very important for post-injury recovery, but are still very poorly investigated, especially in the brain regions located further away from the impact site. The purpose of this study was to investigate possible presence of neuronal damage and appearance of synaptic changes in different regions of the rostral cerebrum within a week after experimentally induced TBI in the rat. Adult male Wistar rats were subjected to TBI of moderate severity using lateral fluid percussion injury (LFPI), induced over the left parietal cortex. Sham-operated rats were used as the control group. Animals were sacrificed 1, 3 or 7 days after procedure and their brains were prepared for histological analyses. Fluoro-Jade B staining was used to detect neurodegenerative changes and immunohistological labeling of synaptophysin (SYP), growth associated protein 43 (GAP-43) and postsynaptic density protein 95 (PSD-95) for determination of neuroplastic responses. Even though neurodegenerative changes were not detected, we observed synaptic changes in some regions of the rostral cerebrum, which varied at different time points and with a different synaptic marker. Our study suggests that TBI causes synaptic changes in regions distant from the impact site, even when there is no obvious neural damage, in the first week following LFPI in the rat.

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## PP8

**B4GALNT1-KNOCKOUT AND CUPRIZONE-INDUCED DEMYELINATION IS ASSOCIATED WITH CHANGES IN PARVALBUMIN AND CALRETININ-EXPRESSING INTERNEURONS IN THE MURINE CORTEX**

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Inhibitory interneurons are key output modulators of cortical micro-columns. Changes in expression of parvalbumin (PV) and calretinin (CR) interneurons have been observed in many neurodevelopmental and psychiatric disorders. We hypothesize that demyelination may also be associated with interneuronal phenotype changes.

The study was conducted on four groups consisting of three male mice each three month old. The control group consisted of C57Bl/6 (wild-type, WT) mice, whereas other groups consisted of WT mice treated with cuprizone, B4Galnt1-knockout mice (KO), and KO treated with cuprizone. The animals were sacrificed at the age of 3 months, their brains were sampled, perfused, cryoprotected and stored at -80°C. Immunohistochemical staining for interneuronal markers PV and CR was performed on free-floating brain sections, and quantification of immunoreactive optical density in hippocampus, caudoputamen, primary somatosensory and primary motor cortices was performed.

Demyelination is associated with statistically significant reduction in PV expression and an increase in CR expression in the murine cortices. Cuprizone-induced demyelination is characterized by quantitative changes of the interneuronal phenotypes, but the general pattern of expression seems to be intact; contrary to that, demyelination caused by the B4Galnt1 gene deficiency is associated with a changed PV and CR expression pattern compared to WT animals. The most profound changes are observed in the somatosensory cortex of the KO animals, whereas their primary motor cortex seems to be unaffected. In cuprizone-fed animals, the phenotype change is most evident in the hippocampus and primary motor cortex.

Demyelination might be associated with phenotype changes of the inhibitory PV and CR-expressing interneurons in the murine cortex. This is especially pronounced among the PV-expressing interneurons, a subpopulation that can be myelinated.

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## PP9

## EXPRESSION ANALYSIS OF A NOVEL NEUROPEPTIDE, UROGUANYLIN, IN THE HUMAN PREFRONTAL CORTEX

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Uroguanylin (UGN) is member of natriuretic peptide family, and upon activation of guanylate cyclase C (GC-C) leads to an increase in cGMP production. GC-C is found in rodent arcuate nucleus of hypothalamus and midbrain where UGN neuromodulates neuronal firing of dopaminergic neurons in substantia nigra and ventral tegmental area by modifying activity of mGluR and mAChR. Previously we showed UGN expression on mRNA and protein levels in several regions of the mouse cortex

The aim of this study was to determine the expression of UGN in the human prefrontal cortex.

Simultaneous detection of UGN mRNA, with calretinin, calbindin and parvalbumin immunofluorescence, was performed with RNAScope technology in the human cingulate and superior frontal gyrus.

Qualitative analysis revealed that UGN mRNA is distributed uniformly through all cortical layers of the human cingulate and superior frontal gyrus. UGN could occasionally be found in white matter as well. UGN is expressed in some calretinin, calbindin, and parvalbumin expressing interneurons.

UGN is expressed in the interneurons of the human cingulate and superior frontal gyrus but we cannot exclude UGN expression in other types of the brain cells. Since we could not determine UGN expression in only one type of the tested interneurons, it raises the possibility of novel interneuron subtype in the human brain.

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## PP10

## SEX SPECIFIC CHANGES IN EXPRESSION OF MAJOR BRAIN GANGLIOSIDES IN THE HIPPOCAMPI OF MIDDLE-AGED RATS ON WESTERN DIET

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Gangliosides are sialic acid-containing glycosphingolipids found on the mammalian cell surface. They are involved in cell-cell interactions and in regulation of signaling pathways. Changes of their expression, because of environmental or genetic factors, might lead to neurological disorders. The aim of this study is to compare the expression of four major brain gangliosides in the hippocampi of healthy and obese diabetic rats. Both male and female Sprague-Dawley rats aged 10 months were included in the study. For 5 months the control group was fed with standard diet and experimental group with food rich in fats and carbohydrates. This type of diet led to obesity and development of type 2 diabetes (T2D). When the animals were 15 months old, the brains were collected and 35 µm-thick cryosections were prepared. The expression of GM1, GD1a, GD1b and GT1b was revealed in the hippocampus CA1 field and dentate gyrus (DG) using immunohistochemistry and was quantified using FIJI. In comparison to control group, the male experimental group had significantly less expressed GM1 within CA1 and DG (p=0.026; p=0.031), while in females its expression level was unaltered. Obese diabetic males had significantly more GD1b and GT1b in CA1 (p=0.001; p=0.0008) and GD1b in DG (p<0.0001). In obese diabetic females noted changes were opposite. There was significantly less GD1a and GD1b in both analyzed areas (CA1: p(GD1a)=0.021, p(GD1b)=0.0006; DG: p(GD1a)=0.0008, p(GD1b)<0.0001), and GT1b in CA1 (p=0.010). High calorie food, and resulting obesity and T2D, affect the expression of major brain gangliosides in sex specific manner. In males the expression of more complex gangliosides increases, but in females decreases.

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## PP11

**INFLUENCE OF LEVEL OF OXYGEN ON EXPRESSION OF MAP1LC3A, A MARKER OF AUTOPHAGY**

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Autophagy is an evolutionary conserved process in which damaged or dead cells and organelles are disassembled and digested by lysosomal enzymes. Although autophagy is normally present at low level, some pathological conditions can induce its activity. Indeed it is shown that in at least some of the neurodegenerative diseases, for example, Parkinson's, autophagy plays an important role in onset and progress of pathophysiological processes. Map1lc3a, microtubule-associated protein 1 light chain 3 alpha is a gene/protein which was recently described as one of the components of autophagy and is therefore used as a reliable marker of autophagy.

The goal of this work was to compare differences in expression of Map1lc3 between concentration of oxygen of 21%, which is condition commonly declared as „normoxic“ and 4% of oxygen, for which we believe that much better mirrors physiological condition during life of neuronal precursors. For this purpose we used neural stem cells obtained from the telencephalon of 14 days old mouse embryos. Cells were cultivated in proliferation medium (DMEM/F12 enriched with B27, N2, FGF2 and bFGF) and then replaced by differentiation medium (Neurobasal) for one day. The levels of Map1lc3a were tested by immunocytochemistry using monoclonal antibody.

When cultivated at 4% O<sub>2</sub>, Map1lc3a was present in a very low number of cells and its expression was hardly visible in perinuclear regions. However, after being exposed to 21% O<sub>2</sub> much stronger and much more common cytoplasmic signal of Map1lc3a was visible. It was seen as granules comparable to the size of small vacuoles. Although a positive signal was visible in a whole cytoplasm, majority of protein was also present in the perinuclear region, typical for autophagosome and/or Golgi.

This result confirms our hypothesis that concentration of oxygen of 21% for neural stem cells represents hyperoxic conditions, to which they react by increasing levels of autophagy.

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## PP12

**SINGLE MODERATE TRAUMATIC BRAIN INJURY IN MICE CAUSES CHANGES IN PSD-95 IMMUNOREACTIVITY IN THE IPSILATERAL CORTEX AND HIPPOCAMPUS**

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Traumatic brain injury (TBI) is the leading cause of death and disability which mostly affects young, work-able individuals. TBI survivors suffer from learning, memory and concentration deficits due to significant cell loss, axonal degeneration and loss of synapses. Postsynaptic density protein 95 (PSD-95) is a scaffolding protein that has a major role in synaptic plasticity.

The aim of this study was to investigate the temporal pattern of PSD-95 immunoreactivity in ipsilateral and contralateral parietal cortices and hippocampi during the first 14 days after experimental TBI.

Lateral fluid percussion injury of moderate severity was induced over the left parietal cortex of adult male C57BL/6 mice. Animals were sacrificed 1, 3 or 14 days after injury and their cortices and hippocampi were prepared for western blot analyses. Brains from another cohort of mice were prepared for immunohistological analyses. Sham-injured animals, used as the control group, were sacrificed 1 day after the sham procedure.

A significant decrease of PSD-95 protein levels in the ipsilateral cortex was evident from day 1 until day 14 after trauma. Double immunofluorescence showed an evident decrease in neuron-specific marker NeuN as well as the loss of PSD-95. In the ipsilateral hippocampus, upregulation of PSD-95 was observed on the day 14 after TBI in regard to sham-injured mice as well as the TBI mice sacrificed 1 and 3 days after trauma. Significant changes in the contralateral cortex and the hippocampus were not observed.

Our preliminary results showed that the cortical levels of PSD-95 are reduced ipsilaterally during the first 14 days after trauma which suggests prolonged synaptic dysfunction in the injured cortex. Upregulation of PSD-95 in the ipsilateral hippocampus points to the presence of synaptic changes in this brain structure.

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## PP13

## CHALLENGES IN COMBINING IN VIVO AND EX VIVO VOLUMETRIC ANALYSIS OF MOUSE ISCHEMIC BRAIN

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Volumetric analysis of the mouse ischemic brain is used in preclinical trials as a tool for monitoring ischemic lesion evolution. In vivo and ex vivo brain imaging provide versatile information on microscopic and macroscopic 3D structure, especially when combining different techniques, for example MRI, x-rays and histology. However, different tissue preparation protocols required for each modality alter the brain morphology and chemical composition, leading to inconsistencies in imaging results, volumetric measurements and biased interpretations. Four groups of C57Bl/6N albino mice underwent a 60-minute cerebral ischemia induced by filament occlusion of the middle cerebral artery. The first two groups underwent different fixation protocols: the Evaporation-of-Organic-Solvent (EOS) dehydration method for synchrotron (SR $\mu$ CT) or micro-CT imaging and a hydrated preparation method for magnetic resonance imaging (MRI). The other two groups underwent different contrast agent staining procedures using phosphotungstic acid or non-ionic iohexol monomer. Volumetric analysis was performed using a manual segmentation method standardized in our laboratory. Brain morphology and ischemic lesion can be easily visualized when using T2-w MR in vivo imaging. However, the signal and tissue contrast decrease after dehydration and fixation. The ischemic lesions were discernible with SR $\mu$ CT ex vivo imaging when the EOS method was applied. PTA staining improved the neuroarchitectonic visualization in SR $\mu$ CT imaging. Volumetric analysis showed that depending on the fixation or staining protocol used the volume of the ischemic tissue can change independently of the surrounding healthy tissue. In vivo MRI enables good gross morphology and lesion area visualization, while micro-CT and SR $\mu$ CT give us high resolution 3D neuroarchitectonic depiction, especially when the tissue is dehydrated or stained. Tissue preparation protocols affect healthy and ischemic tissue in a different manner, leading to volumetric inconsistencies and interpretational biases when doing in vivo/ex vivo multimodal analysis.

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## PP14

## PROLIFERATIVE CAPACITY OF NEOCORTICAL PROGENITORS IS LINKED TO CHANGES IN THEIR MORPHOLOGY

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The evolutionary expansion of the mammalian neocortex is thought to provide a necessary basis for the emergence of human cognitive abilities. Neocortex expansion is determined by the proliferative capacity of neural progenitors and their ability to produce an increased number of neurons during neocortical development. In particular, the proliferative capacity of basal progenitors (BPs) is thought to play a key role. Although molecular differences in BPs of various mammalian species have been extensively studied recently, the mechanistic causes underlying BP proliferative capacity remain largely unknown.

Here, we propose the new concept that BP morphology underlies BP proliferative capacity. By quantifying morphological parameters of BPs in mouse, ferret and human developing neocortex, we found that BPs in human, known to have a high proliferative capacity, also exhibit a high number of processes (cell extensions), whereas BPs in mouse, known to have a low proliferative capacity, accordingly exhibit a low number of processes. To be able to genetically manipulate the morphology of BPs, we applied the CRISPR/Cas9 technology both in vivo in mouse and ex vivo in fetal human neocortical tissue. We found that the morpho-regulatory protein palmdelphin (Palmd) is both required and sufficient for a high number of BP processes. We further show that the reduction in the number of BP processes by PALMD KO in fetal human neocortical tissue results in a decrease in the proliferative capacity of human BPs. At the mechanistic level, we found that a Palmd-mediated increase in the number of BP processes in mouse embryonic neocortex results in activation of integrins and downstream pro-proliferative signaling pathways, leading to an increased proliferative capacity of these cells.

In conclusion, our study provides evidence that cell morphology regulates the proliferative capacity of neocortical progenitors, contributing to the evolutionary expansion of the neocortex.

## PP15

**CHLORPROMAZINE SPECIFICALLY AFFECTS EXPRESSION OF EXTRACELLULAR VESICLE-ASSOCIATED CD81 PROTEIN IN HUMAN GLIOMA AND NEUROBLASTOMA CELL LINES**

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CD81 is a member of tetraspanin family encompassing transmembrane proteins with specific functions in neural cell membranes but also the capability of being secreted into extracellular space as part of extracellular vesicles (EVs). EVs are membrane-enveloped particles with cellular origin and roles in cell-to-cell communication both in physiological and pathophysiological processes. Certain neuroleptic drugs were shown to alter the expression of tetraspanins in animal models and affect EV uptake in vitro. Chlorpromazine (CPZ) is a neuroleptic drug developed for the treatment of schizophrenia. It acts as a dopamine receptor antagonist but can also bind to several other membrane or intracellular proteins. CPZ was found to inhibit clathrin-dependent endocytosis, thereby affecting trafficking of cellular membranes with possible effect on EV biogenesis and uptake by recipient cells. To investigate if CPZ also acts on EV biogenesis, we have examined its effect on the expression of CD81 in neural cells. U-87 MG glioblastoma and SH-SY5Y neuroblastoma cells were treated with growing concentrations of CPZ (4 µg/mL, 8 µg/mL, 16 µg/mL, 32 µg/mL). After 24 hours of treatment, the cells were stained with anti-CD81 antibody and analysed by flow cytometry. Preliminary results have shown that CPZ leads to a significant increase in CD81 expression in U87 cells at the highest dose applied (32 µg/mL). Chlorpromazine downregulated CD81 in SH-SY5Y cells at 8 and 16 µg/mL. In neuroblastoma cells, 32 µg/mL of CPZ resulted in extreme cell loss. These results suggest that CPZ induces changes in CD81 expression with opposite effects on glial cells and neurons. Further studies are needed to link these changes to extracellular vesicle formation and secretion, and thus gain new insight into the effects of antipsychotics on intercellular communication via EVs.

## PP16

**THE EFFECT OF HYPOXIA ON SHAPE AND NUMBER OF MITOCHONDRIA**

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Mitochondria are organelles that play a key role in cell metabolism. Opposite from previous static model, nowadays we know that they exhibit a very dynamic architecture subjected to constant fusion and fission which is in healthy cells in balance. Fusion and fission ratio is depending on oxygen and nutrient availability and all it is dictating mitochondria appearance. The aim of this study was to visualize the influence of ambient and hypoxic conditions on shape and number of mitochondria in mouse neural stem cells.

Neural stem cells were isolated from the telencephalic wall of 14.5 days old mouse embryos, obtained from the C57BL/6 mice strain. Cells were seeded in 24 well plates coated with Poly-D-Lysine and laminine. Seeded cells were subjected to hypoxic (1% O<sub>2</sub>) and ambient (21% O<sub>2</sub>) environment for 24h. Mitochondria were stained with 1mM MitoTracker® Red CMXRos immediately after exposure to different O<sub>2</sub> environment and henceforth incubated at ambient O<sub>2</sub> values environment until fixation. The cells were fixated with 4% PFA 6h and 24h following hypoxia.

Cells fixated 6h after hypoxic exposure exhibited a significant change in mitochondria morphology and they transformed from usual oval and tubular to spherically shaped. 24h of cultivation in normoxia after hypoxic exposure mitochondria regained their normal morphology with respect to control group. When we analysed numbers, 6h following hypoxia exposure the number of mitochondria was reduced, whereas 24h following hypoxia the number of mitochondria increased and values were close to the control group.

These experiments revealed that mitochondria morphology and number in neural stem cells is changing fast in order to adopt to the conditions given by the environment.

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## PP17

### THE EFFECTS OF ACUTE INTERMITTENT HYPERCAPNIA AT DIFFERENT BACKGROUND OXYGEN CONCENTRATIONS ON RENAL SYMPATHETIC NERVE ACTIVITY AND ARTERIAL BLOOD PRESSURE IN RATS

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Acute intermittent hypercapnia (AIHc), as a consequence of repeated episodes of partial or complete upper airway obstructions, is one of the prominent features of obstructive sleep apnea (OSA). Sudden rises in arterial carbon dioxide concentrations activate the chemoreflex pathways which evoke sympathoexcitatory responses. The aim of this study was to examine the effects of AIHc on renal sympathetic nerve activity (RSNA) and blood pressure in presence of different background oxygen concentrations.

The study was conducted on 14 male, urethane-anesthetized, vagotomized and mechanically ventilated Sprague-Dawley rats weighing 280-360 g. Two experimental groups (N=7 per group) were exposed to protocols consisting of five 3-min long episodes of acute intermittent hypercapnia (FiCO<sub>2</sub>=0.15), interspersed either by room air or hyperoxia (FiO<sub>2</sub>=0.5). Mean arterial pressure and RSNA were analyzed in 7 experimental time points: baseline (immediately preceding the first hypercapnic episode), at five hypercapnic episodes and at 15 minutes following the last hypercapnic episode.

AIHc elicited significant activation of RSNA in presence of room air in background, which was attenuated when background hyperoxia was applied (184.7±24.0 % vs. 115.3±6.4 % of baseline, respectively; F=8.51, p=0.013). In room air group, the RSNA response was preserved over all five hypercapnic episodes (F=1.05, p=0.381), whereas in hyperoxia group it was progressively declining towards the last hypercapnic episode (first: 126.0±5.2 % vs. last: 108.8±7.4 % of baseline; F=8.76, p=0.025). At 15 min following the last hypercapnic episode, RSNA remained elevated above baseline in the room air group, whereas in hyperoxia group it was decreased below baseline (122.6±14.5 % vs. 81.0±4.5 % of baseline, respectively; F=7.517, p=0.018). Progressive non-significant increase in blood pressure during exposure to AIHc was observed in both studied groups. High background oxygen concentrations significantly depressed RSNA response to AIHc, suggesting an important contribution of peripheral chemoreceptors in maintenance of the sympathetic response to acute intermittent hypercapnia.

## PP18

### MARKER OF ADULT UPPER CORTICAL LAYERS CUX2 IS EXPRESSED IN TRANSIENT CELL POPULATIONS OF THE HUMAN FETAL BRAIN

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CUX2 is considered to be an adult upper cortical layer marker. Up to now, CUX2 expression pattern in the fetal human cortex is not known, even though human cortex is straightforward to study owing to the extraordinary resolution of laminar developmental events. Due to the subtle subplate (SP) size in rodents, CUX2 is not described in SP in previous developmental studies (Nieto, Zimmer, 2004). However, Kubo et al. (2017) depicted CUX2 during late midgestational human subplate.

Here we followed fetal CUX2 spatio-temporal dynamics in order to get a better understanding of histogenetic interactions during migration, cellular fate commitment, and transient lamination. CUX2 protein expression pattern was studied by immunohistochemistry and immunofluorescence on fixed-paraffin-embedded sections of postmortem human brains from 10 to 38 post-conceptual weeks (PCW), as a part of Zagreb Neuroembryological Collection.

During the pre-subplate (10 and 11 PCW) and subplate formation phase (13 PCW) CUX2 positive cells were present in SP and within the upper third of marginal zone (MZ), evenly spaced in the tangential direction. Furthermore, starting at 15 PCW, we observed CUX2 positive cells in the superficial cortical plate (CP). During the midgestation stage (15-21 PCW) and subplate stationary phase (25 PCW), we observed CUX2+ cells in MZ, CP, and SP; however proliferative VZ and SVZ were not CUX2 positive. Newborn brain (38 PCW) reveals CUX2 positivity in gyral white matter, and scattered cells in MZ of the frontal cortex, as well as distinct subcortical reactivity.

Since CUX2 was present during the pre-subplate stage, when associative neurons didn't migrate through, furthermore it was present in SP during midgestation, and in gyral white matter of a newborn when all associative neurons finished migration, we suggest that CUX2 reactive nuclei belong to postmigratory subplate neurons which are prospective projection (transient associative) neurons. Finally, CUX2 presence in the transient cell populations of developing fetal cortex, and not only in cells destined for upper layers is the reason why it may serve as a reliable indicator of distinct histogenetic events.

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## PP19

### EARLY DIFFERENTIATION OF HUMAN CENTRAL AMYGDALOID NUCLEUS REVEALED BY EXPRESSION OF TRANSCRIPTION FACTOR DLX6

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Amygdala represents a heterogeneous nuclear complex consisting of morphologically different nuclei involved in various functions regarding emotional modulation. Previously, we have demonstrated a human specific transient modular organization of this structure during the midfetal period (Nikolic & Kostović, *Anat Embryol* 1986). Part of a phylogenetically older complex, central amygdaloid nucleus represents the main efferent structure projecting to many brain regions, mainly via stria terminalis and ventral amygdalofugal pathway.

Here we studied possible indicators of the underlying molecular mechanisms in regional differences in maturational processes of this nuclear complex. Reviewing publicly available gene expression database of human brain (Kang et al., *Nature* 2011), several genes were selected which are specifically and highly expressed in human amygdala prenatally. Among them is a transcription factor *Dlx6*, required for molecular differentiation of striatal neurons in developing mouse brain (Wang et al, *J. Comp. Neurol.* 2011).

To investigate *DLX6* expression, we employed immunohistochemistry on fixed-paraffin-embedded sections of postmortem human brains, ranging between 15th and 28th post conception weeks (PCW). The procedure for the human autopsy material was approved and controlled by the Internal Review Board of the Ethical Committee at the School of Medicine, University of Zagreb.

Expression of *DLX6* was visible in the amygdaloid primordiums in the prospective central nucleus, revealing intense nuclear staining. Confocal microscopy analysis using double-staining against NeuN and GFAP revealed that *DLX6*<sup>+</sup> cells colocalized with NeuN<sup>+</sup> but not with GFAP<sup>+</sup> cells, indicating advanced differentiation of central nucleus neurons.

Our results indicate that *DLX6* could be a part of the regulatory molecular program of amygdaloid regionalization, presumably playing an important role in early differentiation of the central amygdaloid nucleus. Since this nucleus represents the main efferent structure, its advanced maturation supports evidence for very early establishment of amygdaloid visceromotor circuitry.

## PP20

### LIPID ENVIRONMENT OF INSULIN AND LEPTIN RECEPTOR IN GLIOBLASTOMA CELLS

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Leptin and insulin regulate energy metabolism at the systemic and cellular level. Cells that express receptors for both hormones are capable of precise sensing of their environment and swift in between metabolic pathways favorable for survival. Also, depending on immediate lipid environment both receptors can be in sensitive or resistant mode. We investigated if glioblastoma cell lines express both receptors and what their lipid environment is.

Seven glioblastoma cell lines were immunohistochemically tested on presence of insulin and leptin receptor. To test glycosphingolipid composition of the membrane we performed lipid extraction and staining with primary antibodies toward complex gangliosides (GM1, GD1a, GD1b and GT1b).

Out of seven lines (CRL1620, H4, SF268, SNB19, SNB75, HTB15 i XF498) two were positive for insulin and leptin receptors and ganglioside GT1b. These cells were treated with sialidase to confirm specific GT1b staining and presence of cell surface sialic acid.

Each of tested cell lines has differently shifted pattern of membrane glycolipids and in our further study we will investigate how that affects activity of leptin and insulin signalling pathway.

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## PP21

**OBSTRUCTION OF MESENCEPHALIC AQUEDUCT AND DEVELOPMENT OF HYDROCEPHALUS**

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Classical concept of cerebrospinal fluid (CSF) physiology assume unidirectional circulation of CSF from site of secretion at choroidal plexuses to site of reabsorption at dural sinuses. Mesencephalic aqueduct which connect III and IV ventricle is „bottleneck“ of this circulation pathway and according to classical concept its obstruction will inevitably led to development of triventricular hypertensive hydrocephalus. Indeed, there are many clinical examples of mesencephalic aqueduct obstruction with associated development of triventricular hypertensive hydrocephalus. However, there are also many clinical cases in which despite mesencephalic aqueduct obstruction no hydrocephalus were observed. In some cases, even after long-lasting follow up there were no sign of hydrocephalus. This cast doubt on a validity and usefulness of classical concept because some clinical cases are just in opposition to the main postulates of classical CSF hypothesis. Our clinical experience showed that in some children with congenital mesencephalic aqueduct obstruction or in patients with aqueduct obstruction due to the large pineal cysts there were no hydrocephalus development as should be expected according to classical concept. According to our studies, mesencephalic aqueduct obstruction will not inevitable led to hydrocephalus and even if hydrocephalus appears this is not due to the blockade of unidirectional circulation but due to the obstruction of bidirectional systolic/diastolic oscillations of CSF through aqueduct. Another important factor in hydrocephalus development is pathology which caused obstruction. If this pathology also alters dynamics of fluids on capillary network this could be another avenue of hydrocephalus development.

## PP22

**NEURODEGENERATION, MICROGLYOSIS AND ASTROCYTOSIS IN THE OPTIC TRACT DURING THE FIRST WEEK FOLLOWING REPETITIVE MILD TRAUMATIC BRAIN INJURY IN WILD TYPE AND TDP-43 TRANSGENIC MICE**

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Repetitive mild traumatic brain injury (mTBI) is associated with the development of neurodegenerative diseases such as amyotrophic lateral sclerosis and chronic traumatic encephalopathy in which TAR DNA-binding protein 43 (TDP-43) dysregulation has been identified. The purpose of this study was to investigate if repetitive mTBI induces neurodegeneration and the changes in microglia and astrocytes in the brain during the first week after the repetitive mTBI in wild type and mice overexpressing mutant TDP-43.

Repetitive mTBI in C57BL/6 and TDP-43G348C (Swarup et al., 2011) mice was induced by using weight drop method (Kane et al. 2012) for five days in a row, twice daily, at intervals of six hours. Sham-injured mice of the control wild type or transgenic TDP-43 mice groups were anesthetized without receiving any injury. The animals were sacrificed 1, 3 or 7 days after the final injury or sham procedure and their brains were prepared for histological analyses. Fluoro-Jade C staining was used to identify the extent of neuronal injury. Immunofluorescence labelings of glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1 (Iba1) were performed with the aim to assess the expressions of astrocytes and microglial cells.

Fluoro-Jade C positive fibers as well as increased GFAP and Iba1 immunoreactivity were detected in the optic tracts of the injured wild type and TDP-43 transgenic mice at days 1, 3 and 7 after the last mTBI.

This preliminary study suggests the presence of neurodegenerative changes as well as of astrocytosis and microgliosis in the optic tract at early time points following repetitive mTBI in wild type and TDP43 transgenic mice.

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## PP23

### AFFERENT CONDITIONING OF MOTOR EVOKED POTENTIALS FOLLOWING TRANSCRANIAL MAGNETIC STIMULATION OF PRIMARY MOTOR CORTEX

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Different types of stimuli (electric and vibration), targeting different afferent sources (cutaneous and muscle) have a range of effects on corticospinal excitability. Mostly the research has focused on effects of electrical stimulation of nerves, cutaneous and mixed, as well as effects of muscle vibration on modifications of magnetic motor evoked potentials (MEPs). Yet, little is known about the effect of cutaneous digit vibration stimuli on motor cortical excitability.

The present study was designed to determine the time course of MEP modulation in hand muscle following cutaneous vibration of hand digit. In eleven healthy subjects, vibration at frequency of 120 Hz was applied to the index finger by means of electromagnetic solenoid-type of stimulation. The protocol consisted of control measurement and paired pulse paradigm where conditioning vibratory stimuli are applied to the digit followed by cortical single TMS pulse over the primary motor cortex at interstimulus intervals (ISIs) of 5-14 ms and of 18-500 ms in two experimental sessions. The results showed striking suppressive effect of MEP amplitudes at ISIs of 200, 300 and 400 ms. This period of late interval suppression is consistent with modifications in the cortical excitability suggested to be due to alterations in excitability of the motor cortex as a result of arrival of the antidromic sensory volley.

## PP24

### AUTOMATED ESTIMATION OF PEAK-TO-PEAK AMPLITUDE AND LATENCY OF MOTOR EVOKED POTENTIALS IN TRANSCRANIAL MAGNETIC STIMULATION STUDIES

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The aim of the present study was to propose an algorithm for automatic analysis of amplitude and latency of single MEP responses recorded continuously in a file. Recorded MEPs often have characteristic shapes and are well defined however, due to intra- and inter-individual MEP variability, any proposed algorithm intended for such signal processing needs to be robust enough to handle signal aberrations. Hence, in order to produce a robust tool for MEP analysis our goal was to deliver an algorithmic solution that can be defined by the following operational modules: 1) extraction from European Data Format (EDF) files; 2) pre-processing (artefacts removal, de-noising); 3) fusion including linking of channel containing MEP responses with the triggering channel data (onset of magnetic stimulation); 4) graphical representation of individual channels; 5) calculation of peak-to-peak amplitudes and latencies of individual MEPs; 6) selection of individual MEP responses for later statistical analysis; 7) graphical presentation of selected MEP signals with basic statistical data; 8) preparing the given data set for future data learning, feature extraction and automatic classification and clustering. The methodology was successfully tested on signals continuously stored as EDF files by means of navigated brain stimulation (NBS) system of the manufacturer Nexstim (Nexstim NBS System 4, Nexstim Oy, Helsinki, Finland). Tests were performed on ten healthy subjects, in a study investigating short afferent cortical inhibition (SAI) by paired-pulse paradigm where conditioning electrical stimuli are applied to the median nerve followed by a single cortical TMS pulse over the M1 at different inter-stimulus intervals (ISIs). The algorithm can serve as additional armamentarium for the analysis of MEPs in research and clinical settings.

## PP25

## MOLECULAR CHARACTERIZATION OF INTERNEURONS IN THE HUMAN PREFRONTAL CORTEX

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Substantial reorganization of cortical network occurring during primate evolution is necessary for the achievement of complex information processing. There is emerging evidence for significant neuronal differences between rodents and human; suggesting distinct membrane and synaptic properties and dendritic complexity of human neurons might contribute to human specific signal processing. Comparative studies indicate that variability in subtle subcomponents of the columnar organization in human and non-human primates, such as the heterogeneous population of the interneuron, are a primary source of interspecies differences. Furthermore, novel research using single nucleus RNA sequencing indicates several interneuron subtypes.

Therefore, the aim of this study was to determine molecular profile of interneurons in human prefrontal cortex. Simultaneous detection of GAD1 (the glutamic acid decarboxylase 67, GAD67) and GAD2 (glutamic acid decarboxylase 65, GAD65) mRNA, and calretinin, calbindin and parvalbumin immunofluorescence, was performed with RNAscope technology. We confirmed that in the human prefrontal cortex vast majority of interneurons express mRNAs for both synthesizing enzymes for GABA, GAD67) and the GAD65. Calretinin neurons can be qualitatively categorized into those who express both, and those who express only GAD67. Calbindin neurons and parvalbumin neurons mostly contain both mRNAs although occasionally neurons without GAD expression are found. In conclusion, even on the level of GABA synthesising enzymes several subpopulations of interneurons can be identified, suggesting possible novel interneuronal functions.

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## PP26

## DENDRITIC REMODELING OF CONTROL AND TUMOR NECROSIS ALPHA DEFICIENT DENTATE GRANULE CELLS FOLLOWING ENTORRHINAL CORTEX LESION IN ORGANOTYPIC TISSUE CULTURES

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Tumor Necrosis Factor-alpha (TNF- $\alpha$ ), a cytokine involved in systemic inflammation, is considered to increase the density of AMPA receptors thereby increasing synaptic strength. TNF- $\alpha$  mRNA levels are increased after denervation in vitro and this helps maintain the homeostatic synaptic response of neurons. In order to investigate how TNF- $\alpha$  affects the morphology of denervated neurons, we used a mouse line lacking TNF- $\alpha$  (TNF-KO) to analyze the changes in dendritic arbors of hippocampal granule cells after denervation in organotypic hippocampal cultures (OTC). Time-lapse imaging of OTCs in Thy1-GFP-TNF- $\alpha$  KO mice was combined with entorhinal cortex lesion (ECL) to investigate the effects of denervation on dendritic arbors of granule cells in vitro. Z-stacks of confocal images of single cells taken at 20 days in vitro (DIV) and DIV 34 were traced and their total dendritic length (TDL) was quantified. In the control situation without ECL, the TDL of dentate granule cells was unchanged between DIV 20 and DIV 34 in both wildtype and TNF-KO control cultures without lesions. Following ECL on DIV 20, TDL was reduced significantly from DIV 20 to DIV 34 in both groups. Denervation of dentate granule cells following ECL leads to a significant, comparable reduction of TDL in both control animals and TNF- $\alpha$  KO mice, indicating that dendritic remodeling after denervation, e.g. caused by traumatic brain injuries, seems to be independent of TNF- $\alpha$  expression in these neurons.

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## PP27

### THE EXPRESSION OF NEUROPLASTIN, CALCIUM ATPase AND SODIUM/POTASSIUM ATPase IN THE BRAIN OF MICE LACKING TOLL-LIKE RECEPTOR 2

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Toll-like receptors (TLRs) are widely expressed in mammalian brain. Involvement in neurodegeneration and hypoxia has been evidenced for several microglial TLRs. In order to investigate interplay of specific microglial and neuronal transmembrane proteins and its potential role in neurodegeneration, we use TLR2 knock-out (KO) mice (C57BL/6 TLR2<sup>-/-</sup>). Our preliminary findings indicate different expression of synaptic transmembrane protein neuropilin (Np) in brain tissue of TLR2 KO mice in comparison with wild-type littermates (WT). Neuropilin, a cell-adhesion molecule found in two isoforms (Np65 and Np55) is an essential auxiliary subunit of plasma membrane Ca<sup>2+</sup> ATPase (PMCA). In addition, Np is influenced by glycosphingolipid environment of the membrane, which is also the case for another ion pumping enzyme, Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA). Since Np, PMCA and NKA all seem to favor specific lipid milieu in order to function optimally, and at least some of them are confirmed to interact with one another, we investigated the expression of these proteins in TLR2 KO compared to WT across different brain regions. We isolated membrane fractions from cortical, hippocampal and cerebellar tissue of 5 WT and 5 KO animals, and analyzed the expression of Np, NKA and PMCA by Western blotting. Data revealed increased expression level of Np isoforms and PMCA in cortex and hippocampus in TLR2 KO, and decreased expression of Np55 and PMCA in the cerebellum. NKA exhibited the same expression pattern in TLR2 KO vs WT, and increased expression in all regions analyzed (cortex, hippocampus, cerebellum). Furthermore, we investigated the cortical NKA activity which is lower in TLR2 KO mice. This data together with ongoing immunohistochemistry and transcriptional analysis will enable more detailed neurobiochemical characterization of TLR2 KO model, and give insight into functional implications of differential regional distribution of here analyzed proteins involved in synaptic plasticity and membrane ion transport.

*This work has been supported by Croatian Science Foundation under the project NeuroReact (IP-2016-06-8636) to SKB.*

## PP28

### CHANGES OF PERINEURONAL NETS MORPHOLOGY, HYPERACTIVE BEHAVIOR AND COGNITIVE DEFICITS IN MILD PERINATAL HYPOXIC BRAIN LESION IN RATS

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In the study, fifty-two Wistar Han® (RccHan®:WIST) rats, (26 females and 26 males) were randomly divided into hypoxic and control group on postnatal day 1 (P1) when hypoxia was induced in a warm (≈ 25°C) hypobaric chamber (Atm 350mmHg, pO<sub>2</sub> 73mmHg) during 2 hours, while controls were kept in normal housing conditions. Behavioral testing were performed at P30 and P70 using open field, hole board, social choice, and T-maze. Samples of brain tissue from adult animals (P105-120) were used for histochemical examination of cytoarchitectonics (Nissl staining), interneurons (parvalbumin immunohistochemistry) and perineuronal nets (Wisteria floribunda agglutinin, histochemistry). Twelve Wistar pups (P1), 6 females and 6 males, was sacrificed at 8, 12 and 24h after hypoxic treatment and samples of their brain tissue were used for examination of microglia (CD68, immunohistochemistry). After mild perinatal hypoxic brain lesion, structural cerebral cytoarchitectonics, as well as the laminar and structural organization of the telencephalon, were preserved. Although, distinct changes in morphology, number, and distribution of the parvalbumin-immunoreactive neurons and perineuronal nets in the midcingulate cortex and hippocampus were observed. Microglial reactivity in lateral ventricle was most prominent at 24h after hypoxia. Compared to controls, motor and socialization patterns were preserved, while treated rats had better performance in open field, especially treated females. Moreover, treated animals also shown impaired learning behavior. Thus, the mild perinatal hypoxic brain lesion in rats leads to consistent disturbances in brain connectivity related to cognitive processes that mimic perinatal mild post-hypoxia condition in humans. Further characterization and evaluation of this non-invasive brain lesion model, on molecular, cytological and connectivity levels, is needed to disclose developmental disturbances that are not compensated after the provoked hypoxia and therefore lead to cognitive deficits.

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## PP29

### DEVELOPMENT AND CHARACTERIZATION OF A NOVEL TRANSGENIC MOUSE MODEL WITH BIOLUMINESCENT AND FLUORESCENT NEURONS FOR IN VIVO IMAGING

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Genetically modified animals are irreplaceable models for studying human diseases. The aim of this study was to generate a transgenic mouse line with bioluminescent and fluorescent neurons, a potentially useful model for studies of brain repair after injury.

DNA plasmid with the desired transgenic cassette was constructed by Golden Gate cloning method. Construct was microinjected into mouse zygotes' pronuclei to generate founders of the transgenic mouse line. Founders (FO generation) were selected among new-born pups and functionally validated using in vivo bioluminescence imaging. Black F1 generation was bred to produce albino F2 generation. The F1 and F2 generations were characterized using in vivo optical imaging, both by bioluminescence and fluorescence modalities.

A vector with a transgenic cassette composed of neurofilament heavy (NFH) promoter and a reporter with fused luc2 (bioluminescent) and FP635 (fluorescent) genes, TurboLuc, was constructed. After successful pronuclear injections, three founder animals were selected. Bioluminescent signal was shown in the founder transgenic animals and two generations of their offspring using in vivo optical imaging. Different expression patterns in the brain and animal body were observed among the F1 generation and three different lines of transgenic males were chosen for further breeding.

In vivo expression of reporter bioluminescent signal in transgenic mice was located in areas where mature neurons can be found. Different mouse lines showed different signal pattern specificity and strength, reflecting the different insertion sites.

Isolated neural stem cells from the novel transgenic mouse lines could be appropriate for research of neural stem cell differentiation, both in vitro and in vivo. Different signal patterns could be a valuable tool in body area-specific nervous system research.

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## PP30

### FATTY ACID TREATMENT AND CHOLESTEROL LOWERING DRUG SIMVASTATIN DISPLACE NEUROPLASTIN Np-65 FROM LIPID RAFTS

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Lipid rafts are cell membrane micro domains enriched in cholesterol, functioning as tightly regulated environment where cell signaling takes the place. Amount of available cholesterol introduces perturbation in lipid rafts composition; affect cell morphology and limits potential for making functional connections to other cells. High fat diet and cholesterol lowering treatment by simvastatin may affect positioning of immunoglobulin synaptic molecules involved in synaptogenesis like neuroplastin Np65.

SH-SY5Y neuroblastoma cell line was differentiated with 10uM retinoic acid and treated with simvastatin, fatty acids (palmitic, stearic and oleic) or combination of both treatments. Lipid raft isolation was carried out by non-detergent method, using 0.5M carbonate buffer, and sucrose gradient with ultracentrifuge separation. Co-localization of lipid rafts markers (Flotillin 1 and GM1) and non-raft marker (Transferrin receptor) with Np65 was determined by western blotting. Western blot quantification was performed in ImageJ program. Total neurite length was measured on 4% PFA fixed cells, stained by cholera toxin and imaged on confocal microscope (Fluoview 1000). Neurites of 50 cells per group were measured in ImageJ program, data are presented as total length of neurite in micrometers.

Cells treated with simvastatin had significantly longer neurites compared to untreated group (49.077±5.59µm compared to 25.57±3.01µm, p<0.001). Also, simvastatin reduced levels of Np65 to 58% of observed in untreated group. Fatty acid treated group had no significant difference in neurite length compared to untreated group, but levels of Np65 were 21.1% of those in untreated group.

Both, simvastatin and fatty acids treatment, displace Np65 from lipid rafts, but have opposite effect on neurite length possible due to different lipid raft perturbation of signalling mechanisms.

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## PP31

### VALIDATION OF SPLIT LUCIFERASE REPORTER SYSTEM FOR THE DETECTION OF HUMAN TAU PROTEIN OLIGOMERIZATION IN LIVING YEAST CELLS

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One of the main neuropathological hallmarks of Alzheimer's disease are neurofibrillary tangles (NTFs), large aggregates of microtubule-binding protein tau that form within the affected neurons. The formation of NTFs is preceded by early-stage tau oligomers, however the molecular pathways that initiate tau aggregation are unclear. To better understand early steps in tau pathology, we constructed a tool for studying tau oligomerization in living cells, based on the luminescent reporter NanoBiT, in which protein-protein interaction results in the complementation of the luciferase NanoLuc, and consequently in generation of luminescence that can be measured in living cells. Since molecular pathways of protein aggregation are largely evolutionarily conserved, we selected a simple cell model, yeast *Saccharomyces cerevisiae*. We separately fused two luciferase subunits to the human tau protein C-terminus. In order to test whether the observed luminescence is a specific result of tau-tau interaction, we constructed negative controls, including cells that carry an empty vector, and cells that express tau fused to only one of the luciferase subunits. Expression of tau constructs was verified using western blot. By measuring luciferase activity in living cells, we showed that cells expressing tau separately fused to two luciferase subunits exhibited an increased luminescence, as compared to controls, indicating the formation of tau oligomers. In order to test whether tau-NanoBiT reporter is able to detect sarkosyl-insoluble tau aggregates, we measured tau-NanoBiT luminescence in *pho85Δ*, *rim1Δ* and *sod2Δ* mutants that were previously reported to have increased levels of sarkosyl-insoluble tau. Our preliminary data showed that the tau-NanoBiT luminescence was elevated in the *sod2Δ* mutant, while the signal in *pho85Δ*, and *rim1Δ* mutants was similar to the wild-type levels. In conclusion, we constructed a luminescent reporter for human tau protein oligomerization in living yeast cells. Our future experiments will address whether tau-NanoBiT reporter correlates with the levels of sarkosyl-insoluble tau aggregates.

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## PP32

### ANATOMICAL SUBDIVISION OF THE SUBTHALAMIC NUCLEI

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Subthalamic nucleus (STN) is one of the clinically most interesting basal ganglia. Neurosurgical stimulation of the STN has helped in treatment of many patients with levodopa resistant Parkinson's disease, but led to some unwanted side effects, which are consequence of stimulating unwanted STN parts. The first study regarding STN division into segments was published in 1925. Since then, 43 studies dealt with this topic in humans and primates using different methods. Although today the popular belief is that STN can be divided into three segments, closer examination of these studies shows that this conclusion is not entirely justified. Only small number of studies advocate three STN segments, while number of segments range from 0 to 4. Due to the extreme clinical significance of the STN and inconsistencies in the description of the number of segments, the aim of this study is to carry out a detailed cytoarchitectonic analysis of the STN and its segments, and to determine whether such segments can be distinguished on magnetic resonance imaging (MRI).

We analyzed the STN section of five adult postmortem human brains without neuropathological changes or clinical signs of prior neurological or mental illness. STN sections were processed using classical cytoarchitectonic as well as modern immunohistochemical methods. Also, 10 healthy participants and Parkinson's disease patients underwent 3T MRI using DBS sequence. MRI analysis of the STN was performed using Analyze 8.1 software. STN histological sections were compared to MR images.

The following architectural parameters were analyzed; the total volume of the STN, total number and density of the STN neuronal displacement and the relative number and distribution of the defined molecular phenotype STN neurons. Based on such analysis, it is possible to determine different segments of the STN, their number and accurate localization. Also, comparison of MRI data between healthy participants and Parkinson's disease patients was done in order to distinguish STN segments.

Better understanding of the STN architectural division and the ability to recognize its architectural divisions on MRI are crucial for more accurate neurosurgical intervention. That allows greater precision and targeted use of stereotactic interventions on the STN, thereby reducing the number of unwanted consequences.

## PP33

**DEEP BRAIN STIMULATION FOR THE EARLY TREATMENT OF THE MINIMALLY CONSCIOUS STATE AND VEGETATIVE STATE**Chudy D<sup>1,2</sup><sup>1</sup>*School of Medicine, University of Zagreb, Zagreb, Croatia,*<sup>2</sup>*Department of Neurosurgery, University Hospital Dubrava, Zagreb, Croatia*

An effective treatment of minimal conscious state (MCS) and vegetative state (VS), caused by hypoxic encephalopathy (HE) or traumatic brain injury (TBI), has not been yet revealed. Several studies with deep brain stimulation of thalamic nuclei in MCS and VS patients were published with most patients after TBI. The aim of our study is to find out the possibility of DBS as a therapy for patients in VS or MCS particularly in earlier phase when the irreversible changes of muscles and joints are not so pronounced. Fourteen patients were included four patients with TBI and 10 with HE. Four of them were in MCS and 10 in VS. Entry criteria included an evaluation neurological status including Rappaport Coma/Near coma scale, electrophysiological status with multimodal evoked potential and 12/24 hours of EEG, and neuroimaging (positron emission tomography and magnetic resonance imaging). The stimulation target was centromedian-parafascicular nucleus complex in the left hemisphere or more preserved hemisphere in patients with TBI. Patients were stimulated daily for 30 minutes every three hours. The parameters of stimulation were as follows: monopolar, intensity to induce "arousal reaction", frequency 25-30 Hz, pulse duration 220  $\mu$ s. Follow up was from 30 to 54 months. Two MCS patients regained consciousness, walking without help, speaking fluently with impressive speech comprehension and no need for assistance in everyday life. One MCS patient reach to the level of consciousness however she is still in wheelchair. One VS patient after ischemic lesion improved to the level of consciousness with possibility of nonverbal communication. Three VS patients died from respiratory infection or sepsis. Other 7 patients, six in VS and one in MCS, remained without substantial improvement of consciousness. For the VS or MCS patients that fulfill clinical, neurophysiological and neuroimaging criteria the DBS of thalamic nuclei could be advised as an option and could be started at rather early stage. We did not figure out neurophysiologic, imaging or clinical marker(s) predicting recovery of patients having very similar features. The studies, which could solve these dilemmas, have to be designed not only using reliable scientific methods but also solving some ethical questions which are specific and more demanding in VS and MCS patients than others.

## PP34

**CORRELATION BETWEEN FUNCTIONAL CLASSIFICATION OF CHILDREN WITH CEREBRAL PALSY AND INTRACRANIAL ULTRASOUND AND MAGNETIC RESONANCE FINDINGS**

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The aim of this population-based study was to evaluate the characteristics of cerebral palsy (CP) and associated impairments in relation to the predominant patterns of the Magnetic Resonance Imaging Classification System (MRICS), as compared to neonatal/early infant cranial ultrasound (CUS).

The study included children from the Surveillance of Cerebral Palsy in Europe (SCPE) C28 RCP-HR – Register of cerebral palsy in Croatia, born 2004-2007. History data, motor functions, accompanying impairments and neuroimaging were evaluated in 227 children with brain MRI, of which 185 also had CUS.

56% of the children had bilateral, 34% unilateral spastic, 9% dyskinetic and 1% ataxic CP. According to the Gross Motor Function Classification System (GMFCS), 62.05% had milder motor impairment (GMFCS I-III) and 37.85% severe (GMFCS IV-V). CUS showed white matter injury in 60%, grey matter injury in 12%, maldevelopments in 8%, miscellaneous changes in 14%, while 6% were normal; MRI showed significant agreement ( $\kappa = 0,675$ ,  $p < 0,001$ ). Neuroimaging findings of maldevelopment and predominant gray matter injury were associated with more severe CP, but 7% of children with CP had normal MRI.

Functional outcomes and accompanying neuroimpairments in children with CP relate strongly to the predominant neuroimaging patterns. The best outcomes were in children with predominant white matter injury. Because of compatibility of CUS and MRI findings, CUS is recommended for children at increased risk of CP. Based on neuroimaging findings, we can predict the type and grade of the neuromotor impairment, that is important for the design and plan interventions and therapeutic procedures, and represents secondary CP prevention.

## PP35

### VOLUMETRIC MAGNETIC RESONANCE IMAGING – BASED MORPHOMETRY AND EARLY MOTOR DEVELOPMENT OF PRETERM INFANTS:

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Brain volume reduction and association between altered volumes and neurodevelopmental outcomes have been reported in prematurely born children. As various cortical and subcortical regions play important role in motor development, local volume reductions might have implications for specific motor outcome. Our aim was to study the relationship between volumetric magnetic resonance imaging (MRI) data in infants born at less than 32 weeks gestation and early motor development at 12 months of corrected age (CA).

Group of forty preterm infants born between 24 and 32 weeks gestational age (GA) were MRI scanned at term-equivalent age (TEA). In addition, volumetric analysis of structural MRI data was performed using semiautomatic software, MNI Toolbox (Montreal Neurological Institute, McGill University, Montreal, Canada). The Infant Motor Profile (IMP) was used for the assessment of early motor development at 12 months of CA.

There was an association between reduced frontal lobe, gray matter and cerebellum volume and low IMP scores. Reduced basal ganglia and thalamus ( $p < .02$ ) and cerebellum ( $p < .04$ ) volumes were significantly associated with poorer motor performance at 12 months of CA.

Reduced volumes of frontal lobe, deep gray matter and cerebellum will affect early motor development, and consequently have role in adverse motor performance at later stages. Our data suggest that volumetric MRI performed at TEA may serve as an additional "biomarker" for motor outcome in preterm infants.

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## PP36

### EARLY NAA AND CHO CHANGES MEASURED BY MAGNETIC RESONANCE SPECTROSCOPY IN DLPC AND AMYGDALA PREDICT LONGER DEPRESSION-FREE INTERVAL UNDER MAINTENANCE ANTIDEPRESSANT TREATMENT

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Brain metabolites quantifiable by proton magnetic resonance spectroscopy (1H-MRS) include N-acetyl aspartate (NAA), considered a putative marker of neuronal integrity and functionality, and choline (Cho), considered as a marker of cellular membrane turnover. Some of previous studies displayed NAA and Cho changes over the individual depressive episode. The aim of this study was to identify 1H-MRS predictors of time to the subsequent depressive episode in patients suffering from the recurrent depressive disorder, who were on maintenance pharmacotherapy, and to analyse mutual correlation of metabolite changes. Changes in NAA and Cho were analysed in DLPFC and amygdala of 48 patients with recurrent depression who underwent maintenance therapy by the judgment of psychiatrist and who continued with the same monotherapy antidepressant treatment during the maintenance period. 1H-MRS evaluations were performed at the beginning of the recovery phase and 6 months later. Patients were clinically evaluated every 6 months and were followed up either to the recurrence of depressive episode or to the start of antidepressant tapering-off. 1H-MRS parameters were evaluated at the start of the recovery phase and 6 months later. Cox proportional hazard analysis was employed to assess neurochemical brain changes as prognostic risk factors for depression recurrence. Five patients were either lost to follow-up or excluded from analysis because of other reasons, so final analysis set consists of 43 patients. Symptoms of depression recurred in 20 subjects. Sustainable NAA and increased Cho levels in dorsolateral prefrontal cortex (DLPFC) at the onset of the recovery phase of the index episode were recognised as markers of antidepressant effectiveness. Patients without recurrent episode had a larger NAA/Cr decrease and a higher Cho/Cr increase in amygdala at the beginning of the recovery phase. Time-varying effect was analysed, and the possibility that the change in parameters is caused by imminent subsequent episode was ruled out. Observed metabolite changes were not mutually correlated in either direction. Opposite NAA direction of change in DLPFC and amygdala accompanied with rise in Cho/Cr, may indicate increased brain resilience in patients who will not experience another depressive episode. These results are consistent with the hypothesis of the alteration in limbic-frontal activity in recovery after depressive episode. Magnitude of observed metabolite changes in DLPFC and in amygdala appear to occur independently in patients with better prognosis.

## PP37

**DEMONSTRATION OF NEUROFEEDBACK TRAINING IN TIC TREATMENT – A CASE STUDY**Kovač D<sup>1</sup>, Golubić S<sup>1</sup><sup>1</sup>*Praedicta, Zadar, Hrvatska*

Tics are stereotypical, uncontrollable and involuntary movements of the body, which can appear in a simple or complex, motor or vocal form. They can be found with 3-10 % of the population and usually disappear by adolescence among 80 % of affected persons. However, the rest of affected population experiences an increase in tic severity. This may affect one's self-confidence and self-image, especially when occurring among younger persons.

Neurofeedback is a kind of biofeedback that changes the brainwaves (EEG waves) with the aim of improving the overall brain functioning and reducing the symptoms which adversely affect a person's mental health.

The aim of this paper is to show tic severity before, during and after Neurofeedback training with two boys of primary school age. Both boys exhibited motor and vocal tics over a number of years, which significantly affected their daily lives. During Neurofeedback training a significant reduction in tic severity was noted (Yale Global Tic Severity Scale - YGTSS), and this condition was retained for as long as 6 months after Neurofeedback training.

## PP38

**MIGRAINE HEADACHES - EFFECTS OF NEUROFEEDBACK TRAINING ON MIGRAINE WITH AURA: A CASE STUDY**Kovač D<sup>1</sup>, Golubić S<sup>1</sup><sup>1</sup>*Praedicta, Zadar, Hrvatska*

Migraine headaches are a serious health problem that directly affects about 10% of the population. One of the common migraine headaches is migraine with aura, i.e. with symptoms preceding the onset of paralyzing pain, which significantly affects daily life.

One of the ways to combat migraine headaches is through neurofeedback. This is a type of biofeedback in which brain waves (EEG waves) are modified with the aim of improving the general functioning of the brain/person and reducing the symptoms impairing their mental health.

This paper presents the results of neurofeedback training with a person suffering from aura migraine for the last 23 years. Significant progress was made in the first 20 neurofeedback training sessions, which resulted in faster recovery after a migraine, a decrease in seizure frequency, and a decrease in their intensity.

Further neurofeedback training resulted in an additional reduction in the frequency and severity of migraine headaches, which remained stable even after neurofeedback training. Also, resistance to common migraine triggers was achieved.

## PP39

### INFLAMMATORY PROCESSES IN DENTAL MEDICINE AND PSYCHOPHARMACOLOGICAL TREATMENT OF DEPRESSION – PRELIMINARY RESEARCH RESULTS

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Interaction of immune system and mental disorders is again in scientists' focus. When considering psychoimmunological issues one should pay attention to inflammatory processes in dental medicine as well. Periodontitis, a chronic inflammatory gingival disease destroying both soft and bone tissues, is linked to antidepressive pharmacotherapy outcome. Though research results are still controversial, based on literature review and our own clinical experience, we hypothesized that periodontitis reduces the probability for positive outcome of major depressive disorder treatment with selective serotonin reuptake inhibitors (SSRIs), independently of other related clinical, vital and sociodemographic parameters.

There were 64 patients who accepted to take part in the study. All relevant ethical principles were applied and respected. Independent variable was the clinical attachment level (CAL) at baseline. CAL was constructed as the sum of periodontal pocket depths (PD) and gingival recession (REC). The primary outcome was the change in Hamilton Depression Rating Scale (HAM-D17) result after three months of treatment with SSRIs. Univariable analysis of change in depression severity during the three-months treatment with SSRIs was calculated.

Statistical significance ( $p < 0,001$ ) in depressive episode severity, meaning symptom reduction after three months' treatment with SSRIs, was observed only in patients without significant periodontitis (CAL value  $< 4.44\text{mm}$ ). At higher CAL values (moderate and severe periodontitis) there is no statistical significance after three months of psychopharmacological treatment.

Preliminary results support the hypothesis that periodontitis reduces the probability for positive outcome of major depressive episode treatment with SSRIs. The result suggests possible role of CAL values as treatment outcome predictors.

## PP40

### VOLUMETRIC INDICATORS OF RECOVERY FROM VEGETATIVE AND MINIMALLY CONSCIOUS STATE AFTER DEEP BRAIN STIMULATION

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Consciousness disorders, which mainly emerge after hypoxic ischemic brain injury (HI-BI) and traumatic brain injury (TBI), are becoming an increasing public health problem. Several studies described the use of deep brain stimulation (DBS) of certain nuclei to regain consciousness in patients in vegetative state (VS) or minimally conscious state (MCS). However, there is a lack of neuromorphological studies on VS and MCS patients who are candidates for DBS.

Ten patients who suffered hypoxic ischemic brain injury or traumatic brain injury were classified as being in VS and MCS, underwent DBS electrode implantation into the centromedian parafascicle complex of the left thalamic intralaminar nucleus, resulting in a consciousness recovery in five of them. Volumetric analysis of pre-DBS magnetic resonance imaging (MRI) scans was conducted for all patients using the CIVET pipeline, automatically calculating volume of grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). Comparison between neuromorphological exams of improved and unimproved patients was conducted.

Results: Differences in pre-DBS volumetric parameters were observed between improved and unimproved group, regarding higher average gray matter absolute and ratio volume and lower cerebrospinal fluid absolute and ratio volume in improved group.

Discussion: Patients who show clear signs of consciousness post-DBS had better preserved brains (more GM, less CSF) pre-DBS, indicating the importance of taking neuromorphological exams of patients obtained prior to DBS into account as possible predictors of recovery after DBS implantation. Possible implications of our results in context of time frame for DBS implantation in patients with consciousness disorders were considered.

## PP41

**DEEP BRAIN STIMULATION IS ACCOMPANIED WITH BRAIN STRUCTURE CHANGES IN VEGETATIVE AND MINIMAL CONSCIOUS STATE PATIENTS**Raguz M<sup>1</sup>, Predrijevac N<sup>1</sup>, Deletis V<sup>2</sup>, Almahariq F<sup>1</sup>, Kostovic I<sup>3</sup>, Chudy D<sup>1</sup>*<sup>1</sup>Department of Neurosurgery, University Hospital Dubrava, Zagreb, Croatia, <sup>2</sup>Albert Einstein College of Medicine, New York, USA, <sup>3</sup>Croatian Institute for Brain Research, Center of Excellence for Basic, Clinical and Translational Neuroscience, University of Zagreb, School of Medicine, Zagreb, Croatia*

Disorders of consciousness (DOC) occur after hypoxic ischemic brain injury (HI-BI) and/or traumatic brain injury (TBI). Several studies described using deep brain stimulation (DBS) of certain nuclei to regain consciousness in patients in vegetative state or minimally conscious state. All those studies missing detail explanation on prospective structural and functional cerebral changes induced by DBS. Therefore, the aim of this study was to analyze region-specific cortical measurements and subcortical structures in order to establish volumetric changes in patient after implanted DBS. Study included 10 patients who suffered HI-BI or TBI resulting in DOC, who underwent DBS electrode implantation into the centromedian parafascicle complex of the left thalamic intralaminar nucleus. Based on consciousness recovery, patients were divided in two groups; non-improved and improved patients. Brain magnetic resonance imaging was obtained at three measuring points; prior to DBS implantation, after DBS implantation, and in improved group, seven years after DBS implantation. Volumetric analysis included changes in gyrification index, regional cortical volume and cortical thickness for parietal, occipital, frontal and temporal lobes, isthmus of cingulate gyrus, parahippocampal and cingulate gyrus and insula. Additionally, subcortical structures segmentation was performed. Limbic cortices, namely parahippocampal and cingulate gyrus and paralimbic cortices, namely insula showed volume increase and present a trend of regional cortical thickness increase in both groups in post-DBS measuring points. Related subcortical structures, namely hippocampus and amygdala were significantly increased in both groups in post-DBS measuring points. In addition, caudate, putamen and accumbens present a trend of volume increase in improved group of patients in post-DBS measuring points. Volume increase in post-DBS measuring points in both groups could be effect of DBS implantation. Our findings confirm the results of previous studies about connection of DBS and short-term brain volume growth, as well as pre-clinical studies of DBS in rodents. In addition, we speculate about long-term effects of DBS. Underlying mechanism of different structural and functional plasticity changes could include axonal remodeling, compensation, overstimulation effect, increase in neuronal size, microvascularization, increase in extracellular matrix, neuritic arborization, even neurogenesis in hippocampus. Regional cortical volume and thickness increase of limbic and paralimbic cortices, as well as related subcortical structures could be indicators of structural plasticity and reorganization in consciousness recovery patients after implantation of DBS.

## PP42

**PREFRONTAL CORTEX ACTIVATION DURING COGNITIVE TASK AS A PREDICTOR OF STRESS RESILIENCE: fMRI vs fNIRS STUDY**Kesedžić I<sup>1</sup>, Božek J<sup>1</sup>, Radoš M<sup>2</sup>, Popović S<sup>1</sup>, Čosić K<sup>1</sup>*<sup>1</sup>Faculty of Electrical Engineering and Computing, University of Zagreb, Zagreb, Croatia, <sup>2</sup>Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Zagreb, Croatia*

Stress resilience is of particular research interest in highly stressful professions, such as first responders, soldiers, pilots, air traffic controllers etc. One of the key brain areas responsible for stress regulation is the prefrontal cortex (PFC), whose activation can be imaged using functional magnetic resonance imaging (fMRI), as well as functional near-infrared spectroscopy (fNIRS). The fMRI is a well-known and complex brain imaging technique, which measures brain activation by detecting changes in the blood-oxygen-level-dependent signal, while the fNIRS is a much cheaper and simpler brain imaging technique, which provides a good insight into PFC activation by measuring the haemoglobin concentration in the PFC. This abstract compares these two techniques and illustrates the potential of fNIRS usage in stress resilience research.

The study included ten right-handed male participants (mean age  $\pm$  SD = 23.14  $\pm$  1.19, the Edinburgh handedness inventory score  $\pm$  SD = 76.35  $\pm$  23.49) imaged using fMRI and fNIRS. Both experiments used a colour-word matching Stroop task that elicits the PFC and enables the measurements and estimations of inhibition, attention, and processing speed.

The results show that the mean level of activation in the PFC using both fMRI and fNIRS is higher in blocks with tasks compared to blocks without tasks, particularly in the dorsolateral PFC. The correlation of the activation between the two techniques, after normalisation across ten participants, is  $r = 0.742$  ( $p < 0.05$ ). A significant correlation was found between a psychological questionnaire measuring stress resilience and the fNIRS task activation ( $r = 0.454$ ,  $p < 0.05$ ). The high correlation between fMRI and fNIRS activation is in line with previous research papers, which indicates that fNIRS, as a simpler and more affordable technique, could be used in a broad spectrum of stress resilience research. The correlation of fNIRS with psychological questionnaire indicates that fNIRS could be used in stress resilience prediction after further research. Although having limitations in spatial resolution, the simplicity of fNIRS recommends its usage in field-deployable and ambulatory applications.

## PP43

### INFLUENCE OF AGE AND GENDER ON COGNITIVE AND PSYCHOMOTOR ABILITIES MEASURED BY THE COMPLEX REACTIONMETER DRENOVAC-SERIES TESTS

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The aim of this cross-sectional study was to examine the influence of age and gender on cognitive and psychomotor abilities measured by tests of the Complex Reactionmeter Drenovac (CRD-series).

A total of 3421 subjects (1427 men) solved three representative CRD-series tests based on chronometry, from the simplest to the most complex one: CRD311 (discrimination of the light signal position), CRD411 (complex psychomotor coordination), and CRD11 (simple arithmetic operations). The total test solving time (TTST), minimum single task solving time (MinT), number of errors, initial dissociation (D1), start (SB), end (EB), and total (TB) ballasts, as measures of wasted time at the first half, second half, and the total test time, respectively, were analyzed.

On CRD11 test, men had shorter TTST than women (134.47±56.43 s vs. 139.17±57.60 s,  $p=0.021$ ), shorter SB, EB, and TB ( $p<0.001$ ), and made less errors than women (2.86 ± 2.61 vs. 3.46 ± 3.33,  $p<0.001$ ). On CRD311 test women had shorter TTST (33.15±7.73 s vs 33.76±8.88 s,  $p=0.033$ ), shorter start, end, and total ballasts ( $p<0.001$ ) than men. On the CRD411 test, men were better than women in all measured variables: MinT (0.49±0.17 s vs. 0.53±0.20 s,  $p<0.001$ ), TTST (40.43±23.29 s vs. 46.33±32.17 s,  $p<0.001$ ), and number of errors (11.26±10.44 vs. 13.01±12.09,  $p<0.001$ ).

On all three CRD-series tests, there were positive correlations between MinT and age ( $P<0.001$ ) and between TTST and age ( $P<0.001$ ), as well as significant increases of D1, SB, and EB with age ( $P<0.001$ ).

Men performed better than women in simple arithmetic and complex psychomotor coordination tests, while women achieved better results in discrimination of light signal tests. Decreased cognitive and psychomotor abilities measured by the CRD-series tests, were associated with advanced age.

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## PP44

### POSTOPERATIVE MONITORING OF COGNITIVE FUNCTIONS AFTER LARYNGECTOMY

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Depending on its stage and localization, laryngeal cancer, apart from issues with respiration, phonation and swallowing, can also cause cognitive impairment. The impairment of cognitive functions is usually followed by the loss of emotional control and changes regarding social relations and motivation. The primary aim in functional surgery of laryngeal cancer is disease control, while the secondary goal is to preserve the basic laryngeal functions. The postoperative period brings challenging adaptation tasks to patients.

The aim of this study was to evaluate the ability to acquire new skills and capabilities, including nasogastric feeding and endotracheal cannula care, as well as achieving self-sufficiency in the postoperative period following laryngectomy by monitoring the cognitive status, mood disorders and the functional status, including the use of the Glasgow Coma Scale (GCS).

This study used database of 88 patients to cTNM (T2-3, N0-2 and M0) and without paraneoplastic syndrom, who had laryngectomy at Department of ENT in period 1/2015-1/2019. Patients were divided into groups according to age: group A (80-86), group B (70-79), group C (60-69), group D (50-59) and group E (40-45).

The inability to acquire new skills was recorded in 75% of group A and 20% of group B. Within group C, 8.7% of patients experienced postoperative delirium (POD), which delayed the start of patient education 4-6 days. The required time to achieve independence after the start of patient education was 7-10 days, with multiple repetitions and demonstrations of the instructions required and reviews of the learned. The time required to adopt new skills in group D and group E was 1-2 days. Loss of laryngeal function consequently caused psychosocial changes in the postoperative period in most of the patients, where age and POD were limiting factors that had significant effects on cognitive functions and the quality of life after surgery.



## PP45

**SYSTEM FOR AUTOMATIC FEATURE EXTRACTION AND PATTERN RECOGNITION IN EEG SIGNAL ANALYSIS**Moštak I<sup>1</sup>, Friganović K<sup>2</sup>, Zelenika Zeba M<sup>2</sup>, Cifrek M<sup>2</sup><sup>1</sup>University of Zagreb, School of Medicine, Zagreb, Croatia, <sup>2</sup>University of Zagreb, Faculty of Electrical Engineering and Computing, Zagreb, Croatia

Electrical activity of the brain recorded with the electroencephalogram (electroencephalographic signals, EEG) can be used for extracting features and identifying certain patterns that best describe explicit human psychophysiological states.

EEG signals are usually analyzed by neuroscience experts in the fields of medical diagnostics and scientific researchers. Manual analysis of EEG signals is a lengthy process and requires vast expert knowledge. Expert experience and subjective impression can significantly influence the analysis. Different preprocessing steps and the choice of removing different artifacts, like blinking or muscle activity, can include risk of bias.

Using MATLAB program package, a system has been developed for automatic EEG signal processing, analysis and feature extraction.

EEG analysis consists of automatic loading of signals and accompanying parameters, semi-automatic removal of artifacts (using Independent Component Analysis, ICA) and the process of separating and calculating features. Features that are included in the developed system are:

changes in the distribution of characteristic EEG signal bandwidth (Power Spectral Density, PSD), spatio-temporal propagation of brain activity (occipitofrontal direction), special brain waveforms like alpha spindle, determination of individual alpha responsiveness interval and individual alpha peak frequency.

Features and repeating patterns that are extracted from the data can be further analyzed in patients with different pathological states (sleep disorders, epilepsy, excessive fatigue, headaches or others). By using automatic signal processing, the analysis is significantly accelerated, and the same criteria for preprocessing and feature extraction is applied to all the EEG signals. Therefore, the likelihood of human error or omission is considerably reduced.

## PP46

**SLEEP, ANXIETY, AND COGNITIVE AND PSYCHOMOTOR ABILITIES OF MEDICAL STUDENTS MEASURED BY TESTS OF THE COMPLEX REACTIONMETER DRENOVAC SERIES**

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The aim of this study is to elucidate the relationship between sleep, anxiety, and reaction times and information processing in solving simple and complex cognitive and psychomotor tasks of the electronic psychodiagnostic test battery, Complex Reactionmeter Drenovac (CRD-series) in medical students.

A total of 168 students (49 men) from School of medicine, University of Split, completed following questionnaires: Sleep Habits questionnaire (SH), Epworth Sleepiness Scale (ESS) assessing daytime sleepiness, Pittsburgh Sleep Quality Index (PSQI) assessing subjective sleep quality, and State-Trait Anxiety Inventory (STAI) assessing anxiety. Cognitive and psychomotor abilities were measured by CRD-series tests: CRD311 (discrimination of the light signal position), CRD411 (complex psychomotor coordination), and CRD11 (simple arithmetic operations), in the sequence from the easiest to the most difficult. In each single test total test solving time (TTST) and minimum single task solving time (MinT) were analyzed. Statistical analysis of the data was performed in MedCalc for Windows, version 11.5.1.0 (MedCalc Software, Mariakerke, Belgium), and significance was considered at  $p < 0.05$ . Comparison of the sleep habits between men and women was done by student's t-test for independent samples. The relationship between CRD test results, sleep and anxiety was calculated by Pearson's correlation coefficient.

Significantly more women reported to be chronically tired (47.1% vs. 24.5%,  $p = 0.007$ ), to sleep less ( $6:35 \pm 1.08$  h vs.  $6:58 \pm 0:58$ h,  $p = 0.024$ ), and to have worse subjective sleep quality ( $p = 0.023$ ) than men. Increased state anxiety was associated with higher alertness ( $r = 0.391$ ,  $p < 0.001$ ), independently of sleep duration. Anxiety as a trait was increased in students with lower perceived sleep quality ( $r = 0.417$ ,  $p < 0.001$ ) and students with excessive daytime sleepiness ( $r = 0.216$ ,  $p = 0.006$ ), both independently of sleep duration. Among investigated variables, only excessive daytime sleepiness, and not the sleep quality, current alertness, nor anxiety correlated with results achieved on CRD tests: with TTST ( $r = 0.168$ ,  $p = 0.033$ ) and MinT ( $r = 0.195$ ,  $p = 0.014$ ) on CRD411 and with MinT ( $r = 0.167$ ,  $p = 0.035$ ) on CRD11 test.

The results highlighted the association between sleep quality, daytime sleepiness, alertness, and anxiety. However, sleep quality, alertness, and anxiety were not predictors of cognitive and psychomotor abilities, which were negatively affected only by daytime sleepiness in medical students.

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## PP 47

**THE INTELLICAGE – AUTOMATED BEHAVIORAL PHENOTYPING IN RODENTS**

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Characterization of animal models of human diseases often faces enhanced data variability due to suboptimal equipment, experimenter interference and animal stress. One way to overcome these problems is the use of automated home-cage based test systems, such as the IntelliCage. Complex behavioral paradigms can be achieved with our IntelliCage system. It allows the evaluation of behavior and cognitive performance of individual mice or rats while they are living in a social group of up to 16 cage mates. This unique principle fosters normal social behavior in an enriched, highly standardized home cage environment, thereby ensuring a high level of animal welfare and minimizing the need for human intervention or single test apparatuses. The IntelliCage decreases the variability of stress reactions in mice and therefore provides consistent data across laboratories. It has been used to transfer standard mouse tests to assess e.g. exploratory behavior, activity, spatial learning, operant/associative learning, memory and animal tests of anxiety. The preprogrammed tasks are evaluated seamlessly by specialized integrated fully automated operant conditioning corners. Each animal is equipped with a RFID transponder/unique tag number and recognized as it enters the conditioning corner. The transponders allow also the selection of specific animals out of the group, via an animal gate the IntelliCage expands to a multi-area system, where fully customizable arenas and mazes can be added to create the PhenoWorld, the behavioral testing standard of the future. Within this poster we introduce the IntelliCage and discuss recent scientific applications and results of the system, which demonstrate reduced data variability and reproducibility.

## PP48

**SOME DETERMINANTS OF WELL-BEING AMONG MOTHERS OF PRETERM INFANTS**

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A normal pregnancy usually ends in childbirth between the 37th and 42nd weeks of pregnancy. A birth that begins before the 37th week is considered premature birth. About 2500 children are born prematurely annually in Croatia, but the public awareness of the problems and consequences that their newborns and their parents face is extremely low. Prematurely born children (the so-called "Palčiči" in our country) are particularly vulnerable in their infant age and during early development, which is why their parents face many challenges. The main aim of this study was to determine the relation of some sociodemographic characteristics of the mother (e.g. mother's age) and the characteristics of childbirth (e.g. gestational age of the child at birth) with some aspects of maternal well-being (stress, anxiety, depression, life satisfaction and post-traumatic growth). The study involved mothers with one child that was born prematurely (N = 161), with an average age of 33 years. The average age of the prematurely born children were 3 years, they were born 2 months before the term, and they were treated on the neonatal intensive care unit on the average of 44 days. Depression, Anxiety and Stress Scale, Satisfaction with Life Scale, Post-traumatic Growth Questionnaire, and Social Support Scale have been applied.

Correlation analyzes showed that measures of age (mother's and child's age) did not correlate significantly with maternal well – being measures, whereas subjective assessment of the mother's own health status was significantly and negatively correlated with some aspects of maternal well-being. That is, mothers who evaluate their own health as well are more satisfied with life and show less symptoms of stress, depression and anxiety. Furthermore, greater satisfaction with life is shown by mothers whose children have no developmental disabilities. When it comes to childbirth characteristics, the gestational age of a child at childbirth is significantly negatively correlated with post-traumatic growth, with higher growth seen by mothers who gave birth to a premature baby with lower gestational age. Accordingly, higher post-traumatic growth is indicated by mothers who have spent more days with their infant in the neonatal intensive care unit.

## PP49

### ROLE OF CONSERVED BIOLOGICAL PATHWAYS AND FUNCTIONING OF MISMATCH REPAIR GENES IN MENINGIOMA PROGRESSION

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Conserved biological pathways such as Wnt signaling pathway and epithelial to mesenchymal transition (EMT) are essential during the embryonic development but can also play a role in tumorigenesis. Resurgence of these pathways with malfunction of p53 tumor suppressor gene and mismatch repair system can give rise to development and progression of tumors. In our research, we studied these cellular events and their functionality in intracranial meningioma – tumors which have three stages of progression, from benign (grade I) to malignant (grade III). By analyzing main effector molecules: p53 (TP53), beta-catenin, E-cadherin (CDH1), MLH1 and MSH2 we can reveal patterns of malignant development and valuable biomarkers of tumor progression. Genetic changes (loss of heterozygosity, microsatellite instability) in TP53, CDH1 and molecules involved in mismatch repair system – MLH1 and MSH2 were analyzed by PCR/RFLP, followed by electrophoresis on Spreadex gels. To assess expression and localization of E-cadherin, beta-catenin and p53, we used DAB-labelled immunohistochemical reaction (EnVision™, Dako REAL™) and monoclonal antibody for each protein. Our results showed that levels of the p53 and beta-catenin were significantly negatively correlated ( $P=0,002$ ) and that the expression of p53 was significantly ( $P=0,021$ ) associated to higher meningioma grades (II and III), indicating that meningiomas with lost p53 upregulate beta-catenin and activate WNT signaling. We also found strong correlation in genetic changes of CDH1 and both MLH1 and MSH2 genes ( $\chi^2=0,007$  and  $\chi^2=0,037$ ), suggesting that loss of function in mismatch repair genes stimulate EMT through loss of E-cadherin.

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## PP50

### NEUROPLASTIN IN THE HUMAN BRAIN

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The cell adhesion glycoprotein neuroplastin (Np, NPTN) has stepped into the spotlight when its genetic variants were associated with the cortical thickness and levels of intellectual abilities in adolescents. Immunohistochemical studies of the human hippocampus revealed that neuroplastin expression delineates the trisynaptic pathway important for episodic memory formation, whereas data from rodent experiments showed that Np antibodies inhibit maintenance of hippocampal long-term potentiation (LTP). Furthermore, genetically-induced deletion of brain-specific neuroplastin isoform causes retrograde amnesia in mice. Structurally, Np is a heavily glycosylated transmembrane protein found in two splice isoforms, neuroplastin-65 (Np65) and neuroplastin-55 (Np55). Np55 is detected in wide range of tissues and has been found in the human breast and lung cancer. However, an adhesive function and a potential role of Np55 in altered cellular proliferation have not been clarified. Np65 is involved in neurite outgrowth, LTP regulation, maintenance of hippocampal CA1 synaptic plasticity, and associative memory formation. It interacts with GABAA receptor subunits in hippocampal inhibitory synapses and regulates ratio of excitatory and inhibitory synapses. Interestingly, data from functional proteomics determined neuroplastin as an essential auxiliary subunit of plasma membrane Ca<sup>2+</sup> ATPase (PMCA), which is one of the key regulators of Ca<sup>2+</sup> signaling and membrane transport. Here we summarize our immunohistochemical data on neuroplastin expression in the human brain during development and its specific immunoreactivity pattern in cell-containing layers of the adult human hippocampus. In addition, we present the transcriptomic expression pattern of neuroplastin and four PMCA isoforms (NPTN, ATP2B1, ATP2B2, ATP2B3, and ATP2B4) in the hippocampus, cerebellum and neocortex from 10 post-conception weeks (PCW) to 82-year of age, analyzed using a previously published microarray database. The high expression level and similar expression pattern of all analyzed genes indicate functional interplay of neuroplastin and PMCA which could underlie specific neuronal functions, e.g. LTP, that rely on ion homeostasis.

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## PP51

### THE ROLE OF INNATE ELECTROMAGNETIC FORCES AROUND NEURONS – IMPLICATIONS ON MICROGLIAL AND ASTROCYTIC ACTIVITY

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Innate electromagnetic fields (iEMF) are fields that are generated by the directed movement of ions through the neuron during an action potential propagation. They are of inhomogeneous nature and exert a force on surrounding neurons and resident cells within the central nervous system (CNS). The aim of our work is to elucidate the importance and action of iEMF and associated forces on activation, function and migration of astrocytes and microglia. Since microglia and astrocytes play a crucial role in development and support of neurons, as well as provide a line of defense from external pathogens, they can also participate in progression or initiation of neuroimmune, neurodegenerative and demyelinating diseases. Like any other cell of the human body, microglia and astrocytes present charged components on their plasma membrane, in the form of ion channels, membrane proteins and various receptors, making them physical point charges – able to be influenced by electromagnetic forces. On top of influencing heat shock proteins, adenosine triphosphate and hypoxia-inducible factor 1 $\alpha$ , innate electromagnetic forces stemming from neurons also possess the ability to influence the ion distribution around the neuronal membrane, as well as calcium ion influx through the voltage gated calcium channels. Our modelling of the innate electromagnetic force provides a novel viewpoint on activation and functional alteration of astrocytes and microglia, demystifying the role of their charged components on disease progression. This work enables us to propose an alternative pathway for microglia and astrocyte activation and provides us with an opportunity to modify treatments to better target the electromagnetic properties of resident cells within the CNS with the purpose of improving patient's condition.

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## PP52

### WNT SIGNALOSOME IS TARGETED IN ASTROCYTOMA

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Wnt signaling pathway plays critical roles in development but also in adult tissue homeostasis, while its abnormal activation is causative for many cancers. Effectors of Wnt signaling: beta-catenin (CTNNB1), AXIN1, DVL1, DVL2, DVL3, TCF1, LEF1, SFRP1 and SFRP3, were investigated in astrocytomas of different malignancy grades. Those most common primary tumors of the central nervous system are classified by WHO into four grades, considering their histology, molecular characteristics and prognosis. PCR/LOH/MSI methods, employing polymorphic microsatellite markers, were used for assessing genetic changes, while protein expressions and localizations were examined by immunohistochemistry. Beta-catenin was upregulated in 50% of glioblastomas and 56% of astrocytomas, while its nuclear location was observed in 52.1% of glioblastomas. AXIN1 was down-regulated in 31% of glioblastomas and 22% of astrocytomas, while LOH of the gene was found only in 10% of glioblastomas. LOHs at all three DVL loci were more frequent in high-grade tumors, especially DVL3 (P=0.007) whose expression also significantly increased with malignancy grade (P<0.001). Contrary, DVL1 expression was downregulated in high-grade tumors (P<0.001). Transcription factors of the pathway, TCF1 and LEF1, were both significantly upregulated. Bivariate correlation of all analyzed proteins showed a statistically significant positive correlation between DVL3 and TCF1 (p=0.020), DVL3 and LEF1 (p=0.006), TCF1 and LEF1 (p=0.021), while DVL1 and DVL3 were negatively correlated (p=0.002). DVL3 (p<0.001), TCF1 (p=0.008) and LEF1 (p<0.001) all showed positive correlation increasing with astrocytoma grades suggesting involvement in malignant progression. Modulators of Wnt signaling, SFRP1 and SFRP3, showed opposing roles. SFRP1 gene was epigenetically silenced in glioblastomas when compared to lower grades (P=0.042), while SFRP3 cytoplasmic expression increased in higher grades. Taken together, our findings reveal important biomarkers influencing astrocytoma prognosis and contribute to a better understanding of its molecular profile.

## PP53

## STEM CELL POTENTIAL IN THE FETAL SUBPLATE

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Cortical neurons are born in proliferative ventricular zone (VZ) and subventricular zone by mitotic division of stem cells and other progenitors, and subsequently migrate to their final destination forming a transient fetal cortical lamination. Our aim was to identify stem cells, as well as cells with proliferative capacity in subplate (SP) zone during midfetal and late fetal period. Herein, the presence of stem cell potential in the human frontal cortex was studied using proliferative marker Ki67, neural stem cell marker SOX2 and selected cell-specific markers. SOX2 maintains the identity of neural stem cells, therefore serves as one of the critical factors controlling neural differentiation. SOX2 is highly expressed in proliferating neural progenitors cells and is downregulated following differentiation into postmitotic neurons or glial cells. During midfetal period, SOX2-positive cells were found in proliferative zones and scattered throughout the SP. In the VZ and SVZ of the frontal cortex, majority of SOX2-positive cells were identified as Ki67-positive, while SOX2 and Ki67 co-localization was found only occasionally in the SP, in addition to uniformly Ki67-positive cells. However, some cells in the SP have only remains of SOX2 reactivity (SOX2+/Ki67-), while sparse SOX2+/Ki67+ reactivity suggests atypically located stem cell. Furthermore, a subset of cells co-expressed SOX2 and GFAP in proliferative zones, but rarely in the SP zone, therefore we cannot implicate if the transitional form of glia in the SP has stem cell potential. Rather, we suggest that remnants of the SOX2+ progenitors move to the SP and reside in yet undefined cell line.

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## PP54

## GENOMIC COPY NUMBER ABERRATIONS FOUND IN ASTROCYTOMA AND THEIR VALIDATION THROUGH cBIOPORTAL DATABASE

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Astrocytic tumors show great heterogeneity in terms of genetic alterations, morphology and behavior. The objective of our study was to discover genomic copy number aberrations (CNA) that are constant across astrocytoma grades, but also those that are specific for progression. In order to identify regions that are driving cancer pathogenesis and novel candidate genes, a cohort of 14 astrocytomas was analyzed by Array Comparative Genomic Hybridization (aCGH) using SurePrintG3 Human microarrays 4x180K (Agilent Technologies). Bioinformatics was performed utilizing Genomic Identification of Significant Targets in Cancer (GISTIC) 2.0.23 and DAVID softwares. Subsequently, the expression data of mRNA, protein and mutations for each annotated gene were explored through cBioPortal web resource providing in silico validation. Altogether, 1438 CNA were found of which losses prevailed. GISTIC identified regions of aberration focusing on 0.25 q-value. Significant deletions affected 14 chromosomal regions, out of which deletions at 17p13.2, 9p21.3, 13q12.11 and 22q12.3 remained significant even at 0.05 q-value. The significantly deleted regions in high grades were: 9p21.3; 17p13.2; 10q24.2; 14q21.3; 1p36.11 and 13q12.11, while amplifications were: 3q28; 12q13.3 and 21q22.3. Low grades comprised significant deletions at 3p14.3; 11p15.4; 15q15.1; 16q22.1; 20q11.22 and 22q12.3 indicating early events. According to DAVID gene annotation software the above regions harbored 65 significantly over-represented genes that were assigned to a pathway or a functional category. Significantly enriched pathways were: PI3K-Akt, Cytokine-cytokine receptor, NOD-like receptor, Jak-STAT, RIG-I-like receptor and Toll-like receptor pathways. Pathways involved in HPV and herpes simplex infections, and pathways involved in inflammation, were also represented. Candidate genes out of 65 over-represented based on Biocportal data were: C1QBP, CCNA1, CHUK, CLDN7, CLEC10A, FGF8, FGF9, FGR, HPS6, NFKB2, PIK3AP1, TRIM8 and YWHAE. Present study brings biologically and functionally significant genetic regions involved in etiology of human astrocytoma which may provide better understanding of the disease for future diagnostics and therapeutics.

## PP55

## THE SEMAPHORIN5A DEVELOPMENTAL EXPRESSION AND FUNCTIONAL ROLE IN MICE TELEENCEPHALON

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The Semaphorin-5A (Sema5A) is a transmembrane protein that has been shown to have a role as bifunctional guidance molecule during axon elongation and cell migration in the development of diencephalon and its connections. Mutations of Sema5A are linked to the etiology of several disorders and syndromes, such as microdeletion of SEMA5A found in patients with autism spectrum and haploinsufficiency for SEMA5A in Cri-du-chat syndrome. We are lacking data on SEMA5A protein expression in human brain but according to the recent data (Allan Brain Institute), the transcripts of Sema5A gene are present in proliferative zones of the developing telencephalon in rodents at E13.5 E15.5 and P14. In this study, in vitro functional assay and post-mortem protein expression analysis were used to reveal the role of Sema5A in brain development in mice. Results show that Sema5A promotes outgrowth and elongation of neocortical axons when uniformly surround immature cortical neurons, but does not steer axons or modulate collateralization. The expression of Sema5A protein was more prominent at P15 than at P5 in the entire cortex with the clear regional differences in the expression with the most prominent expression observed in somatosensory cortex at P15. Further studies of Sema5A in coexpression with specific proteoglycans of extracellular matrix are needed to get a better understanding of the Sema5A role in the development of the telencephalon connectivity in health and disease.

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## PP56

## INABILITY OF TG2576 TRANSGENIC MICE TO COMPENSATE GALACTOSE-INDUCED GLUTAMATERGIC AND ENERGETIC DISRUPTION

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Transgenic models of familial Alzheimer's disease (AD) are predominantly used in AD research, with Tg2576 being a widely used model. We aimed to further characterize this model and analyze its response to oral galactose (Gal), a nutrient that we previously showed to prevent/normalize cognitive and metabolic deficit in sporadic AD. 40 male Tg2576 transgenic mice (TG) and matching wild-type controls (WT) aged 10 months were given oral galactose (200 mg/kg/day; +GAL) or tap water as a drink for two months. At 12 months, mice underwent intraperitoneal glucose tolerance test (ipGTT), and were sacrificed with hippocampal samples withdrawn and frozen. Hippocampal insulin receptor (IR) and phosphorylated/total glutamate receptor (p-NMDAR, NMDAR, p-AMPA, AMPAR) levels were measured by Western blot, glutamate by colorimetric assay, amyloid- $\beta$  (A $\beta$ ) by ELISA.

Compared to WT, TG mice showed impaired glucose tolerance (glucose level increased +16 to +72%), further worsened by Gal exposure ( $p < 0.05$ ). In TG mice, Gal exposure significantly decreased IR (-39% vs TG), glutamate (-44% vs TG, -47% vs WT), p-AMPA (-85% vs TG, -84% vs WT), and NMDAR (-50% vs TG, -58% vs WT) levels but showed no such detrimental effects in WT mice. Conversely, A $\beta$  levels were only increased in the TG+Gal mice group (+59% vs WT,  $p < 0.05$ ). AMPAR and p-NMDAR levels remained unchanged. In conclusion, Tg2576 mice show impaired glutamatergic transmission, likely mediated by A $\beta$ , and inability to correct central and peripheral energetic metabolism disruptions when exposed to oral galactose, in contrast to wild-type mice, which maintain these responses intact.

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## PP57

### NEUROPROTECTIVE EFFECTS OF EXERCISE IN RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE COULD BE MEDIATED BY THE ACTIVATION OF PARAVASCULAR BRAIN WASTE REMOVAL SYSTEM AND PEROXIDASE ACTIVATION

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The paravascular glymphatic system, a regulator of brain nutrient delivery and interstitial waste clearance is one of the key etiopathogenetic factors in neurodegeneration. Exercise has been associated with improved glymphatic flow and amyloid clearance. Intracerebroventricular streptozotocin (STZ-icv) is used to model sporadic Alzheimer's disease (sAD) in rats, but its effects on paravascular amyloid clearance have never been investigated. In this study, 3 months old male Wistar rats (N=36) were treated with STZ-icv or vehicle (3mg/kg STZ/ citrate buffer) and separated in exercise (EXC) and control group (CTR; STZ; CTR+EXC; STZ+EXC). Exercise regimen consisted of 3x3min of rotarod training every second day for 6 weeks. Cognition was examined with passive avoidance (PA) test. Finally, animals were sacrificed, brain was extracted and formalin-fixed paraffin embedded tissue was analyzed with Congo Red polarization microscopy (CRPM), and Thioflavin S (ThS) fluorescence. 3-Amino-9-ethylcarbazole (AEC) was used for endogenous peroxidase assessment. STZ-icv rats were cognitively impaired in comparison with control animals (STZvsCTR=-56%;p<0,05), but EXC prevented cognitive decline (CTRvsSTZ+EXC=-4%;ns). CRPM revealed extensive leptomeningeal and ventricular congophilic deposits in STZ that were not present in CTR, CTR+EXC or STZ+EXC. STZ+EXC reduction in leptomeningeal CRPM signal was accompanied by increased parenchymal paravascular CRPM and ThS fluorescence in the cortical (CTX) and hypothalamic regions (HPT). AEC signal was increased in HPT, CTX and hippocampus only in STZ+EXC. In conclusion, STZ-icv administration induces extensive leptomeningeal congophilic deposition. EXC regimen decreases congophilic deposition likely through activation of paravascular glymphatic clearance and increases protective peroxidase activity.

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## PP58

### CEREBELLUM IN ALZHEIMER'S DISEASE: QUERCETIN AS A POTENTIAL THERAPEUTIC

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The cerebellum is a relatively neglected area of the Alzheimer's disease (AD) brain, probably because it was formerly thought to be spared by the disease. However, a number of pathological changes have now been revealed in the AD cerebellum, principally by immunocytochemical studies, including widespread deposits of diffuse amyloid. Diffuse plaques, also called benign plaques, occur much earlier than neuritic plaques, thus supporting the idea of a therapeutic intervention in the early stage of the disease. The aetiological mechanisms underlying the neuropathological changes in AD still remain unclear but are probably affected by environmental, genetic, and neuroinflammatory factors. This study was aimed to explore neuroprotective role of quercetin as potential therapeutic in the early stage of induced AD. Three-months-old male (n=10) highly inbred Y59 strain rats were intraperitoneally administered with: (a) 0.9% NaCl, HC control group; (b) AlCl<sub>3</sub> (10 mg/kg) and D-(+)- galactose (60 mg/kg), AD group; (c) AlCl<sub>3</sub> (10 mg/kg) and D-(+)- galactose (60 mg/kg) and quercetin (50 mg/kg), AD+Qu50; (d) quercetin (50 mg/kg), Qu50, per day consecutively for 28 days.

The brain samples were prepared according to standard paraffin procedure. Sections of 7-10 microns were stained with hematoxylin-eosin and modified Bielschowsky, comparable areas were analyzed by light microscopy. Changes related to neurodegenerative damage were analyzed by immunohistochemistry using the following primary monoclonal antibodies: purified (azide free) anti-β-amyloid, 17-24 (Clone: 4G8); CD68 (E-11); anti-phospho-PHF-tau pSer202/Thr205 (AT8); anti-tau pSer396/404 (PHF1) and Tau-001 (MC1). Diffuse cerebellar plaques were recognized by antibody 4G8 in AD and AD+Qu50 (reduced number of plaques) group, but neither tau-immunoreactive neurofibrillary tangles nor reactive astrocytes / microglial cells were seen. The results obtained suggest sensitivity of the brain to low doses of aluminium chloride and neuroprotective role of quercetin as a potential therapeutic agent in the early stage of AD.

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## PP59

## DEVELOPMENT OF NON-TRANSGENIC RAT TAUOPATHY MODEL BY APPLICATION OF TAU OLIGOMERS INTO THE ENTORRHINAL CORTEX

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Alzheimer's disease (AD) is the most common secondary tauopathy characterized by progressive loss of cognitive functions and behavioral impairment. The hyperphosphorylation and aggregation of tau proteins progress in a stereotypical manner with the first changes seen in the locus coeruleus and entorhinal cortex from where they spread to the hippocampus and other cortical regions. We aimed to explore whether intracerebral injection of tau oligomers and tau fibrils will induce trans-synaptic spread of pathological tau proteins, and whether those changes would be associated with cognitive impairment. Four-month-old male Wistar rats (n=96) were stereotaxically injected into the lateral entorhinal cortex with tau oligomers, tau fibrils, and phosphate-buffered saline. Animals were analyzed 4, 8 and 11 months post-injection. Cognitive performance was tested using open field, T-maze task, novel object recognition, and object-location test. To specifically detect tau protein changes and perform staging of tau pathology, we used anti-tau antibodies AT8, T22, and HT7.

Immunohistochemistry and immunoblotting of proteins isolated from the entorhinal cortex and hippocampus showed that stereotaxic injection of tau oligomers or tau fibrils into the lateral entorhinal cortex induced phosphorylation of Ser202/Thr205 tau epitope (AT8), as well as HT7-positive (tau aa 159-163) signal present in the brainstem and transentorhinal region. Oligomeric tau was detected with T22 antibody both ipsilaterally and contralaterally to the injection site. Rewarded learning in the T-maze showed slower learning curve with more incorrect choices in rats injected with tau fibrils. We concluded that better understanding of the changes induced by tau oligomers and tau fibrils in this rat model of neurodegeneration has a great potential for revealing mechanisms underlying development and progression of AD and other human tauopathies.

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## PP60

## SEX AND AGE SPECIFIC CENTRAL INFLAMMATION IN RATS UPON CHRONIC STRESS

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Elevated glucocorticoids during chronic stress response are directly connected to central inflammation but the role of aging and sex in such response is still unknown. The aim of this study was to compare the expression of the brain immune cells representatives – microglia and astrocytes by immunohistochemical staining of dentate gyrus (DG) and CA1 hippocampal regions. Male and female rats were divided in young and old animal groups. Chronic stress protocol was performed during 10 weeks. At the ages of 6 and 14.5 months, the brains were collected and dissected for analysis using microglia (Iba1) and astrocyte (GFAP) markers. Experiments were approved under class number 602-04/14-08/06 and registration number 2158-61-07-14-118 in Croatia and under number: CSI/01/3796-7/2015 for the part of the study performed at Faculty of Pharmacy, Szeged. While GFAP expression was lowered in CA1 region upon chronic stress in all animal groups compared to appropriate control groups, expression in DG increased in all except young female stressed group but without reaching statistical significance. Both young and old stressed females revealed significant decrease of Iba1 expression in CA1 and DG upon stress compared to appropriate control groups (p=0.0021 for all comparisons). In young males Iba1 was decreased upon stress in DG (p=0.0021). When young and old stressed males and females were compared, significant decrease was detected in both regions in both female groups upon chronic stress (p=0.0021). While microglia modulate neuronal function during an inflammatory response, astrocytes respond to damage by protecting the hippocampal parenchyma and it seems that microglia activation is strongly sex specific and more important for female stress response.

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## PP61

**SPEECH AND LANGUAGE DISORDERS IN NEURODEGENERATIVE DISEASES – ALZHEIMER'S DEMENTIA AND PRIMARY PROGRESSIVE APHASIA: TWO CASE STUDIES**

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A sharp rise in neurodegenerative diseases has been noted in the world recently. The incidence of patients with dementia has been increasing in Croatia, as well. In here presented case study of a patient with Alzheimer's dementia (AD) and a patient with primary progressive aphasia (PPA), and by comparison of both cases, a preliminary picture of the state of speech-language ability of the participants was obtained. Functions or skills such as understanding, articulation, reading, writing, verbal fluency (phonological and semantic), confrontational naming, semantic and short-term memory were studied. The basic motivation of the work is to gain insight into speech-language skills as well as the development of therapeutic interventions in the treatment of speech-language difficulties caused by the aforementioned diseases. This will provide patients with an additional method of rehabilitation and extend the quality of communication with the family and society.

## PP62

**OXIDATION-REDUCTION CHANGES AND DISTRIBUTION OF TOXIC AND ESSENTIAL/TRACE ELEMENTS IN THE RAT BRAIN INDUCED BY ISOFLURANE AND IRON-DEXTRAN**Odeh D<sup>1</sup>, Oršolić N<sup>1</sup>, Kukulj M<sup>1</sup>, Debić S<sup>1</sup>, Odeh S<sup>1</sup>, Bilandžić N<sup>2</sup>, Sedak M<sup>2</sup><sup>1</sup>*Division of Animal Physiology, Faculty of Science, University of Zagreb, Zagreb, Croatia;*<sup>2</sup>*Croatian Veterinary Institute, Zagreb, Croatia*

Inhaled anesthetics are normally considered non-active, but under appropriate conditions, they are metabolized in reactive free radicals (ROS) and reacting with cellular parts, causing damage. In the brain, iron plays an important role in the production of myelin, metabolism of monoamine transmitters and GABA synthesis. On the other hand, the accumulation of iron in the brain changes brain cell metabolism and leads to increased oxidative stress and neurodegeneration. The aim of this study was to investigate the possible antioxidant/prooxidative effect of isoflurane and Fe-dextran, as well as their combined interaction on the rat brain tissue samples by: a) evaluation of the neuro-inflammation, measuring the relative weight of the brain compared to a healthy control group; b) changes in the oxido-reduction status measuring the level of malondialdehyde (MDA, end product of lipid peroxidation) and glutathione (GSH), activity of catalase (CAT) and superoxide dismutase (SOD); c) NO analysis as well as distribution of essential and toxic metals by mass spectrometry inductively coupled plasma (ICP-MS). The results indicate that isoflurane causes oxidative stress in the brain, while its combination with Fe-dextran as well as Fe-dextran itself causes increased lipid peroxidation and neuro-inflammation. Because of the increased levels of oxidative stress and the formation of superoxide anions, the level of NO in the iron dextran treated group and its combination with isoflurane decreased due to the formation of peroxynitrite which as an intermedator for protein oxidation, lipid peroxidation, mitochondrial dysfunction causes apoptosis and necrosis respectively neurodegeneration. Furthermore, the results of ICP - MS indicate a statistically significant increase in toxic metals (Al, Pb) as well as essential elements in brain tissue (Fe, Zn) in the rats treated with iron dextran and combination of iron dextran and isoflurane compared to the control group. The biggest changes in the distribution of toxic and essential trace elements / elements are seen in the group treated with iron dextran and isoflurane compared to other groups. The results indicate that the iron dextran and the combination of iron dextran and isoflurane have a neurotoxic effect through increased lipid peroxidation, ROS, which ultimately leads to cell death and degeneration of brain tissue.

## PP63

**EXPRESSION OF THE ACTIVATED MICROGLIA CELLS MARKERS IN ALZHEIMER'S DISEASE**

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Numerous studies have documented that uncontrolled immune response in the brain contributes to the progression of Alzheimer's disease (AD). Although microglia cells should be protective, pathological changes cause aberrant and stronger microglial activation. Uncontrolled microglial response leads to the constant production of pro-inflammatory factors, severe inflammation and oxidative stress which becomes detrimental for neighboring neurons. Therefore, the main goal of this study was to analyze the expression of microglia cells markers in the different areas of the hippocampal formation in the brain of subjects with Alzheimer's disease and control samples. We will also compare markers for elevated inflammation in the cerebrospinal fluid (CSF) of AD subjects with non-AD subjects as controls. Markers of microglial activation are detected with immunohistochemistry method. Different areas of hippocampal formation are tested for the expression of microglia cell markers and compared with numbers of amyloid plaques and neurofibrillary tangles in the same hippocampal regions. The research has shown that the expression of microglia cell markers differs in the AD brains and controls. We found a stronger microglial activation in the AD subjects and higher expression of the markers in the areas of hippocampal formation most affected by AD pathology. These results support the inflammatory hypothesis of neurodegenerative diseases and suggest that the aberrant microglial response could be essential for the development and progression of degenerative changes in AD.

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## PP64

**EVIDENCE FOR DECREASED DENSITY OF CALRETININ-IMMUNOPOSITIVE NEURONS IN THE CAUDATE NUCLEUS IN PATIENTS WITH SCHIZOPHRENIA**Adorjan I<sup>6</sup>, Bin S<sup>1,4,5</sup>, Feher V<sup>3</sup>, Tyler T<sup>3</sup>, Veres D<sup>6</sup>, Chance SA<sup>2</sup>, Szele FG<sup>1</sup>

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Schizophrenia (SCH) and autism spectrum disorder (ASD) share several common aetiological and symptomatic features suggesting they may be included in a common spectrum. Recent results suggest that excitatory/inhibitory (E/I) imbalance is relevant in the aetiology of SCH and ASD. Numerous studies have studied this imbalance in regions like the ventromedial and dorsolateral prefrontal cortex. However, relatively little is known about neuroanatomical changes of subcortical structures, such as the caudate nucleus, in neuropsychiatric disorders. We recently showed significant decrease in calretinin-immunopositive (CR-ip) interneuronal density in the caudate nucleus of patients with ASD. CR-ip neurons constitute more than 50% of caudate interneurons and are likely crucial for maintaining E/I balance. Therefore here we examined the immunohistochemical distribution of calretinin and NPY-immunopositive neurons in the caudate nucleus and the dorsolateral prefrontal cortex. The state of microglial activation was assessed by quantification of Iba1- and TMEM119-immunopositive cells.

There were small, medium and large CR-ip interneurons detected in the caudate nucleus. We found a 38% decrease in the density of all CR-ip interneurons that was driven by the loss of the small CR-ip interneurons in patients with SCH. The densities of the medium and large CR-ip and of the NPY-ip interneurons were not significantly altered.

The discovered changes in caudate nucleus interneuronal populations suggest that function of caudate is impaired in schizophrenia. Our results warrant further studies focussing on the function of CR-ip neurons and on the caudate nucleus as a possible hub for information selection and regulation of associative cortical fields whose function may be altered in SCH.

## PP65

## ANALYSIS OF THE MEF2C TRANSCRIPTION FACTOR EXPRESSION IN DEVELOPING HUMAN CINGULATE GYRUS

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The Myocyte Enhancer Factor 2C (MEF2C) is a transcription factor expressed in brain exclusively in neurons exiting the cell cycle and during their final differentiation. Data collected in studies on rodents have revealed that MEF2C might be implicated in upper-layer neuron specification and excitatory synapse differentiation. The human brain transcriptome study (Kang et al., 2011) showed upregulated MEF2C mRNA during the midgestational period (19-24PCW) in the anterior cingulate cortex (ACC), which is implicated in neurodevelopmental disorders such as autism, intellectual disability, and schizophrenia. The revelation of the temporal pattern of MEF2C protein expression during human fetal and perinatal period in the ACC and also in other brain regions could be important for the understanding of normal and pathological cortical development. The automatic analysis of the neuronal immunohistochemical (IHC) staining is fast, objective, and provides consistent and unbiased results obtained in all sections. This method, already used in studies with adult brain samples, is here used in fetal brain samples. Fetal human brain samples from Zagreb Neuroembryological Collection, staged 21 gestational weeks (GW) to 3 postnatal months, stained for MEF2C and neuronal marker NeuN were used for light microscopy analysis, whole-slide scanning (Hamamatsu NanoZoomer 2.0 RS) and automatic neuron detection method in order to disclose the dynamics of MEF2C protein expression in the ACC. The neuron locations are obtained automatically and used for cell density distribution analysis. MEF2C immunoreactivity (ir) was found in the ACC from 26 GW onward, with changing maturational patterns of expression throughout the ACC and other cortical regions. Comparison with the NeuN-ir was made in order to compare the specificity of MEF2C staining. Aiming to discover specific neuronal subtypes and cortical layers expressing MEF2C, specific multiple labeling on more brain samples should be studied.

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## PP66

## PREFRONTAL CORTEX IN NEURODEVELOPMENTAL DISORDERS: INSIGHTS FROM THE ORGANIZATION OF BRODMANN AREA 10 IN WILLIAMS SYNDROME

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The prefrontal cortex encompasses a number of areas underlying higher order cognitive and emotional processing. Its frontopolar part – Brodmann area 10 (BA 10) – often displays compromised structure in neurodevelopmental and psychiatric disorders. Of particular interest is its lower (magnopyramidal) layer III, underlying long cortico-cortical connections between BA 10 and other areas in the cortex. Among the disorders of particular interest that show differences in the organization of prefrontal cortex compared to controls is Williams syndrome. The disorder is associated with a specific deletion in the chromosomal band 7q11.23~25 and characterized by a distinct behavioral phenotype, including hypersocial behavior, which suggests involvement of higher order cortical areas underlying social cognition. Here, we present an analysis of layer III in BA 10 in Williams syndrome, with the focus on the distribution and soma size of neurons staining positive for non-phosphorylated epitope of neurofilament protein (SMI-32ir neurons). Our results suggest that two aspects of SMI-32ir neurons differ between Williams syndrome and neurotypical individuals: decreased density of SMI-32ir neurons in the lower layer III of BA 10 and smaller size of their cell bodies. Given that Williams syndrome is characterized by an overall decrease in density of neurons in BA 10, our results suggest that the decrease may be driven by the decrease in the density of SMI-32ir neurons and that lower layer III may be particularly affected. The present research carries implications for understanding cortico-cortical connectivity between BA 10 and other cortical areas underlying social behavior in Williams syndrome and may be relevant to other disorders affecting the prefrontal cortex.

## PP67

**TESTING PRESENCE OF DYSKINETIC EYE MOVEMENT DISORDER IN CHILDREN WITH DYSKINETIC CEREBRAL PALSY**Ivošević M<sup>1</sup>, Alimović S<sup>2</sup>, Moslavac A<sup>3</sup>, Bošnjak Nađ K<sup>4</sup>, Mejaški-Bošnjak V<sup>5</sup><sup>1,2</sup>Faculty of Education and rehabilitation Sciences, University of Zagreb, <sup>3,4</sup>Special hospital for protection of children with neurodevelopmental and motor disorders Goljak, Zagreb, <sup>5</sup>Children's Hospital, Medical School, University of Zagreb

Despite the fact that ocular and cerebral abnormalities are shown to be very frequent in cerebral palsy (CP), children with CP are underreferred to rehabilitation services for visual impairments. Visual component is, together with the motor disorder, an integral part of the clinical picture of CP and not an associated symptom. Therefore, an accurate detection of visual disorders and visual function not only lead to a complete clinical diagnosis but also to an appropriate intervention plan. Hence, the need for a study aiming specifically to describe all the aspects of visual involvement in the dyskinetic CP. Research goals were aimed at gaining insights into the nature of visual impairments and functional vision of children with dyskinetic CP, determining the nature of connection between visual functions and functional vision, with an emphasis on searching for dyskinetic eye movement disorder for understanding the difficulties in performing visual activities of two children with dyskinetic CP from the Zagreb's county register of CP, which is part of national C28 RCP-HR-Register of cerebral palsy of Croatia included in Surveillance CerebralPalsyEurope(SCPE). The data were collected using standardized tests for visual function assessment (direct and indirect pupil reaction, eye position, position and stability of monocular corneal reflex, eye motility, visual acuity, contrast sensitivity, visual field). Dyskinetic eye movement disorder was tested comparing the tested results of visual functions. Functional vision (solving close-up vision tasks, communication and interaction, orientation and mobility and activities of daily living) was tested through the observation of the children's behavior and through open structured questions addressed to parents. Cerebral visual impairment was examined by a questionnaire for the screening of cerebral visual impairment. Qualitative research analysis shows which ocular and cerebral visual impairments are present as well as their relation to visual functioning. Moreover, it shows clinical features of dyskinetic eye movement disorder that haven't been shown present among tested children. Since this is the second research up to this date, testing an eye movement disorder that specifically occurs in dyskinetic CP. It's characteristics are further discussed and defined. In contrary to previous study, in a child where highly inefficient visual functioning was shown, the cause goes wider from the abnormal eye motility. It consists of combination of several motor and sensory problems (lacking binocular visual acuity, contrast sensitivity, fixation, voluntary eye movements and oculomotricity). GMFCS level, proper head and body positioning, environmental as well as children's psychological components play a significant role in overall assessment of visual functioning. Due to the small sample and findings that are, on account of heterogeneity of dyskinetic CP hard to compare, future research is needed to expand overall knowledge of functional vision and visual functions needed for planning rehabilitation and education management for children with dyskinetic CP.

## PP68

**ETHANOLIC EXTRACT OF PROPOLIS EXACERBATES COPPER-INDUCED NEURONAL DEATH: THE INVOLVEMENT OF ROS/P53/P38 INTERACTIONS**Jazvinščak Jembrek M<sup>1,2</sup>, Vlainić J<sup>1</sup>, Radovanović V<sup>1</sup>, Hanžić N<sup>1</sup>, Oršolić N<sup>3</sup><sup>1</sup>Ruđer Bošković Institute, Zagreb, Croatia, <sup>2</sup>Catholic University of Croatia, Zagreb, Croatia, <sup>3</sup>University of Zagreb, Faculty of Science, Zagreb, Croatia

Increased levels of copper are considered as contributing factor in the progression of neurodegenerative diseases by promoting oxidative stress conditions. The aim of our study was to examine effects of ethanolic extract of propolis (EEP) against copper-induced death in cultured P19 neuronal cells. Propolis is a heterogeneous resinous product collected by honey-bees. Due to its well documented health beneficial effects, including antioxidative and neuroprotective, propolis and its extracts are widely used in traditional and modern medicine. Surprisingly, we found that small, per se non-toxic concentrations of EEP exacerbated copper-induced death of P19 neurons. The neurotoxic effect of small concentrations of EEP was particularly pronounced in severe oxidative stress. In the presence of excess copper EEP increased production of reactive oxygen species (ROS) and stimulated caspase-3/7 activity. EEP also promoted copper-induced increase in the expression of transcription factor p53 and its downstream target Bax. The detrimental effects of EEP were accompanied with a prominent up-regulation of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a metabolic enzyme that may sense oxidative stress and participate in cell death events. SB203580, an inhibitor of p38 mitogen-activated protein kinase (MAPK) completely prevented toxic effects of EEP in severe oxidative stress conditions, whereas SP600125, an inhibitor of c-Jun N-terminal kinase (JNK), demonstrated a significant pro-death effect. Our data indicates a pro-oxidative and apoptotic mode of EEP action in the presence of excess copper, and suggest an important role of ROS/p53/p38 crosstalk in death cascade. Although propolis is considered as a safe natural remedy, the obtained results pointed out that caution is required during its prolonged consumption in certain medical conditions. Our study also emphasized that detailed pharmacological and toxicological studies must be carried out for propolis and other natural dietary supplements in order to identify the entire repertoire of their undesirable side-effects.

## PP 69

### INTRODUCING THE SLOPE OF THE OXYGEN DESATURATION CURVE AS A NOVEL INDEX IN ASSESSING PHENOTYPES IN SEVERE OSA PATIENTS

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Apnea-hypopnea index (AHI) is the main parameter used to diagnose and assess severity of obstructive sleep apnea (OSA), but different phenotypes of OSA exist among patients with similar AHI. Therefore, the slope of the oxygen desaturation curve (Slope Index, SI) might be relevant as a novel parameter in OSA severity and phenotypes assessment. The aim of this study was to investigate the relationships between SI and AHI with daytime sleepiness, sleep efficiency and comorbidity presence in severe OSA patients.

A total of 111 patients who underwent whole-night polysomnography (PSG) at the Split Sleep Medicine Centre with severe OSA ( $AHI \geq 30$ ) were included. Among PSG parameters AHI and sleep efficiency were analyzed. Daytime sleepiness was assessed by Epworth Sleepiness Scale (ESS). Arterial hypertension, diabetes mellitus type 2 (DM2) and depression were self-reported. The SI was calculated as the averaged quotient of the difference between the blood oxygen saturation before and after the obstructive apnea episode and the duration of the episode throughout the night.

Higher ESS score was associated with both increased SI and increased AHI in non-hypertensive ( $r=0.406$ ,  $p=0.004$ ;  $r=0.497$ ,  $p<0.001$ , respectively), non-diabetic ( $r=0.274$ ,  $p=0.001$ ;  $r=0.309$ ,  $p=0.003$ , respectively) and non-depressive ( $r=0.206$ ,  $p=0.043$ ;  $r=0.251$ ,  $p=0.014$ , respectively) severe OSA patients. In severe OSA patients with hypertension and severe OSA patients without depression, sleep efficiency was positively associated with SI ( $r=0.260$ ,  $p=0.045$ ;  $r=0.232$ ,  $p=0.022$ , respectively), but not AHI ( $r=0.155$ ,  $p=0.242$ ;  $r=0.079$ ,  $p=0.445$ , respectively). In patients with DM2 sleep efficiency was positively associated with AHI ( $r=0.550$ ;  $p=0.012$ ), but not SI ( $r=0.257$ ,  $p=0.261$ ).

Both indices, SI and AHI, predicted daytime sleepiness in severe OSA patients without comorbidities. SI better predicted sleep efficiency in severe OSA patients with hypertension, as well as in the patients without depression. The slope of the oxygen desaturation curve index, as a novel OSA severity index, could help in understanding the impact of hypoxia in different OSA phenotypes.

## PP70

### POLYSOMNOGRAPHY PARAMETERS AND SLEEP ARCHITECTURE: THE ROLE IN DAYTIME SLEEPINESS AND SLEEP QUALITY OF OSA PATIENTS

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The aim of the current research is to evaluate significant contribution of sleep stages distribution and polysomnography determined sleep variables to excessive daytime sleepiness and sleep quality in OSA patients.

Participants recruited for this study were 500 patients aged over 18 years, referred to the Split Sleep Medicine Centre. All participants attended a whole-night polysomnography and completed the following questionnaires: Pittsburgh sleep quality index (PSQI) and Epworth sleepiness scale (ESS). Linear regression was conducted with ESS and PSQI score as dependent variables. Mild OSA was diagnosed if AHI ranged from 5 to 15, moderate OSA in AHI from 15 to 30, and severe OSA in AHI above 30. Polysomnography parameters model included sleep latency, wake after sleep onset, total sleep time and slow-wave sleep, whereas Sleep architecture model included percentage of time spent in stage N1, N2, N3 and REM stage.

Polysomnography parameters model have low predictive value for sleep quality ( $R^2=2,4\%$ ;  $p=0,022$ ) and for excessive daytime sleepiness ( $R^2=4,5\%$ ;  $p<0,001$ ) in patients with all stages of OSA severity. However, the assessment in specific OSA severity groups resulted in different results. While of low predictive value, daytime sleepiness is still significantly predicted based on polysomnography parameters only in mild ( $R^2=7,1\%$ ;  $p=0,039$ ) and moderate ( $R^2=13,5\%$ ;  $p=0,010$ ) OSA, whereas no significant prediction was possible in severe OSA. Sleep stages remained non-significant in the prediction of daytime sleepiness, even in specific OSA severity groups. However, sleep quality is significantly predicted based on Sleep architecture model ( $R^2=9,4\%$ ;  $p=0,004$ ) and Polysomnography parameters model ( $R^2=7,8\%$ ;  $p=0,026$ ) only in mild OSA.

Polysomnography parameters model predicts daytime sleepiness in mild and moderate OSA patients, but not in severe OSA patients. However, despite of OSA severity, daytime sleepiness is not predicted based on Sleep architecture model. Sleep quality is dependent on sleep stages distribution and polysomnography parameters only in mild OSA.

## PP71

**SHORT LATENCY AFFERENT CORTICAL INHIBITION IN OBSTRUCTIVE SLEEP APNEA SYNDROME: A TMS STUDY**Rogić Vidaković M<sup>1</sup>, Jerković A<sup>1</sup>, Šoda J<sup>2</sup>, Vujović I<sup>2</sup>, Benzon B<sup>1</sup>, Đogaš Z<sup>1,3</sup>

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Patients with obstructive sleep apnea syndrome (OSAS) show repetitive upper airway obstructions that occur during sleep and are allied with a reduction in blood oxygen saturation resulting in intermittent hypoxia, sympathetic excitation and sleep fragmentation. OSA is associated with the range of medical conditions such as hypertension, obesity, type 2 diabetes, depression, as well as with psychological and cognitive deficits. However, neurophysiologic mechanisms of OSAS are complex and still obscure. Preliminary evidences of Nardone et al. (Sleep Medicine 2016; 24:51-56) point to dysfunctions in cholinergic system assessed by short afferent cortical inhibition (SAI) technique. The SAI phenomena is thought to depend on neural interactions within the cerebral cortex either by inhibition of the motor cortex from fast conducting afferents or via withdrawal of tonic facilitation from thalamic structures.

The objective of this exploratory study was to investigate SAI in severe OSAS patients (AHI >30 h<sup>-1</sup>) by paired-pulse paradigm where conditioning electrical stimuli are applied to the median nerve followed by cortical navigated (nTMS) over the primary motor cortex for hand muscle representation. Magnetic resonance images (MRI) of the head were obtained to suit the TMS requirements and were integrated in the nTMS system and used for the 3D reconstruction of individual's brain. Motor evoked potentials (MEPs) were recorded from the abductor pollicis brevis (APB) muscle with nTMS conditioned by electrical stimuli applied to the median nerve at the wrist at interstimulus intervals (ISIs) of 18 to 28 ms in a group of seventeen severe OSAS patients, and twelve healthy control subjects. Univariate analysis was performed to identify the differences on SAI between the control group and OSAS patients. The results from this study indicate significant reduction on SAI in severe OSAS patients when compared with controls. The role of SAI as potential marker in early detection and treatment of OSA patients is still to be examined in future studies.

## PP72

**SLEEP ARCHITECTURE IN SEVERE OSA PATIENTS**Valić M<sup>1,3</sup>, Valić Z<sup>2</sup>, Pecotic R<sup>1,3</sup>, Lusic Kalcina L<sup>1,3</sup>, Pavlinac Dodig I<sup>1,3</sup>, Dogas Z<sup>3</sup>

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Obstructive sleep apnea is a disorder characterized by repeated cessations of breathing during sleep. Consequences of OSA are related to changes in sleep architecture with decreased amount of deep sleep stage N3 and REM sleep. Protrusion of alpha activity into sleep represents a non-restorative sleep in clinical settings. Sleep spindles are a hallmark of sleep stage N2 implicated in multiple brain functions including sleep quality, sensory gating, learning and memory. This study was performed to investigate sleep architecture, and distribution of alpha brain activity and sleep spindles in severe OSA patients.

Polysomnographic data of 99 patients with OSA referred to Split Sleep Medical Center in Split, Croatia, EU, were analyzed for parameters including sleep duration, percentage and duration of sleep stages N1, N2, N3 and REM, count of alpha and spindles in N2 according to Neuro Report on Alice 5 device. Two groups of OSA patients with no other comorbidities (OSA-nocm) were compared: severe OSA-nocm (N=31), and mild OSA-nocm (N=8). Additionally, group of severe OSA patients with comorbidities (hypertension and/or diabetes mellitus, OSA-hdm, N=60) was analyzed and compared to severe OSA-nocm group.

Severe OSA patients had decreased amount of N3 compared to patients with mild OSA (3.6±41% vs. 10.0±6.3%, p=0.01). Alpha count in N2 was increased in severe compared to mild OSA-nocm patients (665.8±499.6 vs. 288.3±292.5, respectively, p=0.03). Alpha count in severe OSA-hdm patients was 1705.88±2373.78 whilst in severe OSA-nocm was 665.8±499.6 (p=0.215). Sleep spindles count in N2 in severe OSA-nocm patients was not significantly different in comparison to mild OSA-nocm patients (279.1±437.9 vs. 186.8±239.1, p=0.207).

Decreased amount of N3 sleep stage and increased alpha activity during N2 sleep stage indicated impaired sleep architecture and non-restorative sleep in severe OSA patients, accentuating the effects of OSA on sleep architecture

## PP 73

## COMBINING IN VIVO IMAGING MODALITIES IN EVALUATION OF MOUSE BRAIN AFTER ISCHEMIC LESION

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Small laboratory animals serve as an essential tool to evaluate the future medical interventions in particular for brain diseases, where neuroprotective or neurorestorative treatments are still elusive. The clinical relevance of the mouse models of the human ischemic stroke represents a key element of eventual translation to clinics. Temporary occlusion of medial cerebral artery (MCAO) followed by the reperfusion is accompanied by substantial variability of the lesion and development of outcomes through time. Subsequently, the in vivo imaging represents a solution to monitor and evaluate the consequences of the brain ischemia. Medial cerebral artery occlusion (MCAO) for 60 minutes was followed by reperfusion. The ischemic lesion was evaluated by magnetic resonance imaging (MRI, Bruker 7T Biospec 70/20 USR) and bioluminescence imaging (BLI, Perkin Elmer IVIS Spectrum), complemented by functional tests and Western blot. Tlr2, Casp3 and 7, and Gap43 were used as molecular markers. Tlr2 loss of function induced modified neuroinflammation.

The bioluminescence imaging was used to follow the expression of Tlr2 as neuroinflammation marker and Gap43 as plasticity/restoration marker. The innovative caged-luciferin based bioluminescence imaging was applied for assessment of Casp3 and 7 activity to evaluate apoptosis. When Tlr2-deficient group with modified neuroinflammation was compared to control (wt mice) group the differences were revealed using multimodal approach only. Combining bioluminescence and MRI allowed for standardization of the measurements according to the size of ischemic lesion. This revealed the significant increase of elements of repair and apoptosis in the tested group with modified neuroinflammation. When bioluminescence imaging is combined with magnetic resonance, the resulting multimodal approach allowed to assess the elements of brain repair after ischemic lesion in the mouse.

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## PP74

## EFFECTS OF NEONATAL NORMOBARIC HYPOXIA ON RAT BEHAVIOR AT YOUNG AND ADULT AGE

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Prenatal hypoxia is one of the main causes of neonatal hypoxic-ischemic encephalopathy which can result in a wide range of consequences, from severe mental retardation, cerebral palsy, and epilepsy to milder learning difficulties and behavioral disorders. Studies on rat models are necessary for understanding the behavioral outcomes of hypoxic brain injuries of different intensities, and their molecular basis. Our research group is trying to develop a non-invasive model of rat neonatal hypoxia, which corresponds to human prenatal hypoxia in midgestation (23-32 weeks of pregnancy). The aim of this study was to determine possible changes in locomotion, learning, anxiety-like, exploratory and social behavior in young rats neonatally exposed to normobaric hypoxia, as well as their persistence in the adulthood.

On the first postnatal day (PND1), experimental pups (36 Wistar rats, both sexes) were kept under hypoxic conditions, which were induced in a warm ( $\approx 25^{\circ}\text{C}$ ) normobaric chamber (8% O<sub>2</sub>, 92% N<sub>2</sub>) during 2 hours. Controls (40 Wistar rats, both sexes) were kept in normal housing conditions. Rats underwent the battery of behavioral tests: open field, hole-board, T-maze and social choice from PND33 to PND45. 17 control and 15 hypoxic rats were retested from PND70 to PND82.

In comparison to the control group, young hypoxic rats displayed highly significant increase in the number of rearings in the open field test and highly significant decrease in the number of nose pokes in a hole board test, indicating increased alertness and neophobia in an open space. Adult rats still had significantly increased number of rearings, but there were no differences in exploratory behavior. Our results suggest that neonatal exposure of rats to decreased oxygen levels may induce behavioral impairments at young age, represented as increased aspects of anxiety-like behavior which, in a milder form, persist in the adulthood.

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## PP75

## THE ROLE OF GUANYLATE CYCLASE-C ON ISCHEMIC STROKE

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Stroke has been identified as one of the leading causes of mortality in industrialized countries. Natriuretic peptides are involved in different physiological and pathophysiological conditions in the brain. Agonists of guanylate cyclase A but not B lead to decrease of brain lesion size after middle cerebral occlusion (MCAO). Therefore, uroguanylin (UGN) and its receptor guanylate cyclase C (GC-C), could also play a role in development of brain lesions and edema after ischemic injury.

The purpose of this study is to describe the role of GC-C and its agonist, UGN, in the development of ischemic stroke in the murine model of ischemia.

MCAO was performed in wild type (WT), GC-C and UGN knock-out (GC-C  $-/-$ , UGN  $-/-$ ) mice. Lesion volumes were measured 1 and 7 days after stroke and correlated to neurological impairment test scores.

GC-C  $-/-$  animals show a significant reduction in lesion volumes 1 day after MCAO compared to their WT counterparts, whose lesion volume diminishes 7 days after MCAO. However, UGN  $-/-$  animals developed similar lesion volumes and oedema size 1 day after MCAO as their wild-type littermates (UGN  $+/+$ ).

**CONCLUSION:** Our results show that the activation of GC-C could lead to an increase in lesion volume following ischemic stroke. The difference in stroke volume is visible 1 day after MCAO, and is only present in GC-C  $-/-$  and not in UGN  $-/-$  animals. This difference could indicate GC-C activation by another agonist or the existence of an GC-C independent signalling pathway.

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## PP 76

## MATURATIONAL CHANGES OF WHITE MATTER SEGMENTS IN PRETERM CHILDREN REVEALED BY FA AND ADC VALUES

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White matter (WM) lesions are the most important pathogenetic substrate of neurological abnormalities in prematurely born infants. WM organization into the white matter segments (WMS) is most precisely described by Constantin Von Monakow, where each segment contains different commissural, projection and association axonal pathways. Exact location and extent of lesion is often difficult to detect by conventional magnetic resonance imaging (MRI), especially considering subtle lesions. Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) are MRI diffusion parameters often used in assessment of WM abnormalities. The aim of this study was to evaluate whether diffusion parameters measured in each WMS can show maturational and eventually pathological changes in preterm children at term equivalent age (TEA) and at second year of life. Infants scanned TEA were divided in groups by birth time (22-28 postconceptional weeks-PCW, 28-32PCW, 33-36PCW + control group), and by presence or absence of visible lesions on MRI. Examinees scanned at 2nd year of life were also divided into the groups using the same criteria. Most important results show that: 1) there are significant differences in FA values between all WMS in both time points except segments III and IV at 2nd year; 2) FA values show obvious decrease in radial direction (ventricles  $\rightarrow$  pia); 3) different groups divided by birth time significantly differ among each other considering FA values while those differences diminish or even disappear in toddlers; 4) ADC values are more sensitive to discern pathological/normotypic group compared to FA values. In conclusion, FA and ADC values expressed in relation to WMS give us insight in WM differentiation and can serve as additional criteria for both maturation level and topography of developmental lesion. In addition, we have demonstrated protracted WM maturation which seems to be one of the key features of human brain development.

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## AUTHOR INDEX

<b>A</b>			<b>C</b>			<b>Đ</b>							
Adorjan I . . . . .	97	Caratan S . . . . .	72	Đogaš Z . . . . .	50, 56, 57, 76, 79, 102, 103, 104, 105								
Alimović S . . . . .	100	Cesarec Augustinović S . . . . .	82	<b>E</b>									
Almahariq F . . . . .	65, 74	Chance SA . . . . .	97	<b>F</b>									
Anticevic A . . . . .	27	Chudy D . . . . .	65, 66, 73, 74	<b>E</b>									
August I . . . . .	99	Cifrek M . . . . .	78	<b>F</b>									
Aurer A . . . . .	72	Cuevas D . . . . .	99	<b>F</b>									
<b>B</b>			<b>Č</b>			<b>G</b>			<b>I</b>				
Babić Leko M . . . . .	92, 96	Čičak K . . . . .	77	<b>G</b>			<b>I</b>			<b>I</b>			
Babić Perhoč A . . . . .	89, 90	<b>Ć</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bajs Janović M . . . . .	69	<b>Ć</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bakarić R . . . . .	87	Ćosić A . . . . .	43	<b>G</b>			<b>I</b>			<b>I</b>			
Balog M . . . . .	93	Ćosić K . . . . .	75	<b>G</b>			<b>I</b>			<b>I</b>			
Banovac I . . . . .	34, 35	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bardak A . . . . .	93	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Barić A . . . . .	36, 46	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Barkovich AJ . . . . .	22	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Benzon B . . . . .	56, 57, 104	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bičanić I . . . . .	37	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bilandžić N . . . . .	95	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bin S . . . . .	97	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Blažetić S . . . . .	41	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Blažević A . . . . .	38	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Boban M . . . . .	64	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bobić-Rasonja M . . . . .	88, 98	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bokulić E . . . . .	39	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Borovečki F . . . . .	87	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bošnjak Nađ K . . . . .	100	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Božek J . . . . .	75	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Branović Čakanović K . . . . .	91	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Brkić AL . . . . .	36	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Brlek P . . . . .	82, 85	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
Bukovac A . . . . .	82, 85, 87	<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
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		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
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		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
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		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
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		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
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		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		
		<b>D</b>			<b>G</b>			<b>I</b>			<b>I</b>		

Petrović D ..... 98  
 Pilipović K ..... 40, 45, 55  
 Popović S ..... 75  
 Predrijevac N ..... 73, 74  
 Puljko B ..... 60  
 Pušar D ..... 88

**R**

Radmilović M ..... 36, 60  
 Radonić E ..... 72  
 Radoš M . . . 38, 54, 69, 75, 109  
 Radovanović V ..... 101  
 Raguž M . . . 38, 65, 68, 73, 74,  
 82, 109  
 Rajič Bumber J ..... 55  
 Rakic P ..... 31  
 Rašin MR ..... 24, 35  
 Ratko M ..... 108  
 Rietsche M ..... 59  
 Rogić Vidaković M 56, 57, 104  
 Rojnić Kuzman M ..... 34  
 Rončević A ..... 43  
 Rosenzweig I ..... 30  
 Russig H ..... 80

**S**

Savić A ..... 69  
 Sedak M ..... 95  
 Sedmak D 34, 35, 38, 42, 58  
 Sedmak G . . . 39, 65, 83, 98  
 Semendeferi K ..... 99  
 Sestan N ..... 20  
 Skokic S ..... 46  
 Smilovic D ..... 59  
 Sotošek Tokmadžić V .... 48

Spajić S ..... 49  
 Srakočić S ..... 62, 106  
 Stipan M ..... 107  
 Stojanović M ..... 60  
 Strbačko I ..... 54  
 Szele FG ..... 97  
 Szűcs K ..... 93

**Š**

Šalković-Petrišić M . . . 89, 90  
 Šarac H ..... 69  
 Šečić A ..... 69  
 Šimić G . . . 64, 83, 91, 92, 96  
 Šimic Klarić A ..... 67  
 Škokić S ..... 36, 106, 108  
 Šoda J ..... 56, 57, 104  
 Šošarić B ..... 91  
 Španić E ..... 92, 96  
 Štajduhar A ..... 98, 109

**T**

Tomas D ..... 85  
 Tot M ..... 77  
 Trnski S ..... 61, 88, 107  
 Tromba G ..... 46  
 Tyler T ..... 97

**V**

Valenta M ..... 62  
 Valić M ..... 50, 76, 79, 102,  
 103, 105  
 Valic Z ..... 105  
 Vasung L ..... 25  
 Veres D ..... 97  
 Vidaković M ..... 81

Viljetić B ..... 41, 53  
 Vlahović D ..... 91  
 Vlainić J ..... 101  
 Vujović I ..... 56, 57, 104  
 Vukšić M ..... 52, 59

**Z**

Zelenika Žeba M ..... 78  
 Zima D ..... 88  
 Zjalić M ..... 41, 43, 63  
 Zrinščak I ..... 91  
 Zubčić K ..... 64

**Ž**

Živko M ..... 54  
 Žunić Išasegi I ..... 68, 109  
 Župan G ..... 40, 45, 55  
 Župan Ž ..... 40



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