SNC'11

SiNAPSA Neuroscience Conference '11

Central European FENS Featured Regional Meeting

Ljubljana, Slovenia, September 22-25, 2011

SiNAPSA NEUROSCIENCE CONFERENCE '11

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Book of Abstracts

www.sinapsa.org/snc11

Cankarjev dom, Ljubljana, Slovenia
22—25 September 2011

Sinapsa Neuroscience Conference ‘11
Central European FENS Featured Regional Meeting

Organized by SiNAPSA, Slovenian Neuroscience Association

Programme Committee
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IBRO, International Brain Research Organization
EC-IFCN, International Federation of Clinical Neurophysiology
ARRS, Slovenian Research Agency
Faculty of Medicine, University of Ljubljana
University Medical Centre Ljubljana

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	Thursday, September 22nd	**Friday**, September 23rd	**Saturday**, September 24th	**Sunday**, September 25th
		Registration	Registration	Registration
8:00		**Plenary talk** LD Colin Blakemore	**Plenary talk** LD Richard Frackowiak	**Parallel symposia** LD Lifetime cortical circuitry reorganization KD Amygdala networks and fear
9:00		**Parallel symposia** LD Mechanisms in epileptogenesis KD Structure and function of cortical circuits	**Symposium** KD Neuronal ensemble recording LD EWAN: Neural bases of emotion	
10:00		Coffee	Coffee	Coffee
11:00	Faculty of Medicine **Young Neuroscientist Forum** **Ljubljana 2011**	**Parallel symposia** LD Unconventional EEG use: TMS, BCI KD Interneurons in the Neocortex	**Symposium** KD Intercellular signaling in DA dysfunctions LD EWAN: Emotion and mood in psychopathology	**Parallel symposia** LD Sex differences in brain and behaviour KD Endocannabinoids and plasticity
12:00				**Plenary talk** LD Barry J. Dickson
13:00		**Poster session A**	**Poster session B**	
14:00				
15:00		**Parallel symposia** LD Skeletal muscle disorders mechanisms KD Working memory: role of interference	**Symposium** KD AD information system breakdown LD EWAN: Clinical cases, specific topics and contraversial issues	
16:00	**AOŽ Memorial lecture** LD Palmer Taylor	Coffee	Coffee	
17:00	OPENING OF THE SNC'11 CE FFRM	**Plenary talk** LD Luiz Pessoa	**Plenary talk** LD Maria Grazia Spillantini	CLOSING OF THE SNC'11 CE FFRM
18:00	**Plenary talk** LD Pasko Rakic	**EJN Best Publication Award Talk** LD Lea Siksou	**Dr. Janez Faganel Memorial Lecture** LD Donald B. Sanders	
19:00	Neuroscience and Society Dialogue **Ethical dilemmas in neuroscience** panel discussion	**Translation neuroscience** LD Drug induced neuroplasticity in the PFC FENS	**Translation neuroscience** LD Dementias: from biomarkers to drugs	
20:00	Welcome reception **Wine and cheese by the posters**	**Guided tour of the old town**	Conference dinner **FENS SiNAPSA Social at the castle**	
21:00				

Linhart Hall LD
Kosovel Hall KD

EWAN:
Educational workshop on affective neuroscience

FENS:
Meet the FENS (Štih Hall)

Lecture Halls Map

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SiNAPSA Neuroscience Conference '11

Programme

www.sinapsa.org/snc11

Cankarjev dom, Ljubljana, Slovenia
22—25 September 2011

SiNAPSA Neuroscience Conference '11 Programme

Thursday, 22 September

14:00—20:00 **Registration** | Second Foyer

16:00—17:00 Andrej O. Župančič Memorial Lecture | Linhart Hall
The α,β-hydrolase fold: offering adhesion and catalysis within the synapse
Palmer Taylor

17:00—17:30 **Opening of the SiNAPSA Neuroscience Conference '11** | Linhart Hall

17:30—18:15 Plenary talk | Linhart Hall
Principles and mechanisms of neuronal migration: relevance for human brain disorders
Pasko Rakic

18:15—20:00 **Neuroscience and Society special event: Ethical dilemmas in neuroscience** | Linhart Hall
Panel discussion

20:00—22:00 **Welcome reception: Wine and cheese by the posters** | Second Foyer

Friday, 23 September

7:30—19:00 **Registration** | Second Foyer

8:30—9:15 Plenary talk | Linhart Hall
Neural plasticity: liberating the brain from its genetic constraints
Colin Blakemore

9:15—10:45 Symposium | Linhart Hall
Molecular mechanisms in epileptogenesis
Chairs: Günther Sperk and Merab Kokaia

The role of changes in the GABA system and of endocanabinoids in epileptogenesis
Zsofia Magloczky

The role of neurogenesis in the development of epilepsy
Merab Kokaia

A role for leukocyte-endothelial adhesion mechanisms in epilepsy
Paolo Fabene

Over-expression of GABA system in the dentate gyrus of patients with temporal lobe epilepsy patients indicates a protective mechanism
Günther Sperk

9:15—10:45 Symposium | Kosovel Hall

Structure, plasticity and function of cortical circuits

Chair: Simon Rumpel

Short and long term structural plasticity in the mouse neocortex

Anthony Holtmaat

Timing of GABAergic neurons in cortical circuits

Thomas Klausberger

Dynamics of the mouse auditory cortex

Simon Rumpel

Information transformation between cortical regions during a tactile task

Mathew Diamond

10:45—11:00 **Coffee break** | Second Foyer

11:00—12:30 Symposium | Kosovel Hall

Interneurons in the neocortex

Chairs: Christian Wozny and Gábor Tamás

Self-inhibition of fast-spiking basket cells: functional roles within cortical microcircuits

Alberto Bacci

Frequency-dependent inhibition between neocortical pyramidal cells

Gilad Silberberg

Interneurons at the end of the functional spectrum

Gábor Tamás

Functional roles of neurogliaform cells in superficial layers of the neocortex

Christian Wozny

11:00—12:30 Symposium | Linhart Hall

Unconvential use of EEG: TMS co-registration and BCI

Chair: Paolo Battaglini

Combining EEG and transcranial stimulation in neuroplasticity studies

Carlo Miniussi

TMS-EEG studies of brain excitability and connectivity

Risto Ilmoniemi

Brain-computer interfaces: basic principles and perspectives

Paolo Battaglini

Brain-computer interfaces: muscle-independent communication for the paralyzed

Tamara Matuz

12:30—14:30 **Poster session & Lunch** | Second Foyer

Molecular neuroscience A

Systems neuroscience A

Cellular neuroscience A

Clinical neuroscience A

Cognitive neuroscience A

History of neuroscience

Computational neuroscience

14:30—16:00 Symposium | Linhart Hall

Molecular mechanisms of selected skeletal muscle disorders

Chair: Salvatore DiMauro

Mitochondrial diseases of the skeletal muscle

Salvatore DiMauro

Regulation of the sodium pump in skeletal muscle in conjunction with metabolically altered conditions

Alexander V. Chibalin

Effect of neural agrin on the regenerative potential of the human skeletal muscle

Paola Lorenzon

Response to hypoxia in the in vitro regenerating human skeletal muscle

Sergej Pirkmajer

14:30—16:00 Symposium | Kosovel Hall

Subcomponents in working memory: the role of interference

Chair: Jure Bon

Emotion-cognition interactions in schizophrenia: effects of emotional distraction on working memory

Alan Anticevic

Concurrent TMS-fMRI investigations to provide direct evidence for top-down prefrontal control in the presence of external interference

Eva Feredoes

Working memory function in neurodegenerative disorders: a lesson from Huntington's disease

Christian Robert Wolf

Interference control in visuospatial working memory

Jure Bon

16:00—16:15 **Coffee break** | Second Foyer

16:15—17:00 Plenary talk | Linhart Hall

On the relationship between emotion and cognition

Luiz Pessoa

17:00—17:45 EJN Best Publication Award 2011 | Linhart Hall
A common molecular basis for membrane docking and functional priming of synaptic vesicles
Lea Siksou

17:45—19:15 Translational neuroscience | Linhart Hall
Drug-induced neuroplasticities in the prefrontal cortex
Chair: Ronald E. See

Psychostimulant-induced neuroadaptive changes in prefrontal cortex and cognitive dysfunction
Ronald E. See

Modulation of the neurotrophin BDNF in prefrontal cortex by psychotropic drugs
Fabio Fumagalli

Neuroadaptations in amygdala and prefrontal cortex produced by antidepressants
Nina Karpova

The role of cortical mGluRs in methamphetamine-induced memory deficits
Marek Schwendt

18:00—19:00 **Meet FENS** | Štih Hall

FENS contribution to member National Societies, Advocacy programme
Sten Grillner

FENS organization: FENS Committees and their activities
Fotini Stylianopoulou

FENS Forum 2012 in Barcelona
Ole Kiehn

European Journal of Neuroscience
Jean-Marc Fritchy

20:00—21:00 **Guided tour of the old town**

Saturday, 24 September

7:30—19:00 **Registration** | Second Foyer

8:30—9:15 Plenary talk | Linhart Hall
Classifying clinical images: a new aid to dementia diagnosis with implications for treatment strategies
Richard Frackowiak

9:15—16:00 **Educational Workshop on Affective Neuroscience** | Linhart Hall

9:15—10:45 Symposium | Kosovel Hall
Neuronal ensemble recordings – insights into the function and dysfunction of brain circuits
Chair: Laszlo Acsady

Ensemble activity of topographically aligned relay and reticular cells in the thalamus
Péter Barthó

Using neural ensemble recordings to elucidate circuit dysfunction in the Parkinsonian basal ganglia
Peter J. Magill

Encoding and reactivation of spatial memory traces by hippocampal cell assemblies
Jozsef Csicsvari

Structure of neuronal population activity in auditory cortex
Kenneth D. Harris

10:45—11:00 **Coffee break** | Second Foyer

11:00—12:30 Symposium | Kosovel Hall
Intracellular signalling mechanisms of dopamine related dysfunctions
Chairs: Gilberto Fisone and Riccardo Brambilla

Identification of neuronal and molecular targets for antiparkinsonian and antipsychotic drugs
Gilberto Fisone

The Ras-ERK signalling pathway in the control of hyperdopaminergic disorder
Riccardo Brambilla

Pharmacological and genetic modulation of signalling pathways improves L-dopa induced dyskinesia: RGS, GRK and PSD-95
Erwan Bezard

Experimental models of dopamine-related dysfunctions
Raul R. Gainetdinov

12:30—14:30 **Poster session & Lunch** | Second Foyer
Clinical neuroscience B
Cognitive neuroscience B
Molecular neuroscience B
Systems neuroscience B
Cellular neuroscience B
Neuroscience methods

14:30—16:00 Symposium | Kosovel Hall
Breakdown of the superinformation system in Alzheimer disease: culprits and victims
Chair: Michal Novak

Classification and basic pathology of Alzheimer disease
Charles Duyckaerts

The fatal dialog between chronic neuroinflammation and tau neurodegeneration
Norbert Zilka

Are tau proteins only microtubule-associated proteins? Toward a role in nucleus and plasma membrane
Luc Buee

Neuroendocrine – immune interactions in the pathogenesis of neurodegeneration
Peter Filipcik

16:00—16:15 **Coffee break** | Second Foyer

16:15—17:00 Plenary talk | Linhart Hall
Untangling the role of protein aggregation in neurodegenerative diseases
Maria Grazia Spillantini

17:00—17:45 Dr. Janez Faganel Memorial Lecture | Linhart Hall
Biomarkers for myasthenia gravis
Donald B. Sanders

17:45—19:15 Translational neuroscience | Linhart Hall
Dementias – from detecting biomarkers to designing drugs
Chair: Zvezdan Pirtošek

Alzheimer's disease: present and future treatment strategies
Bengt Winblad

Candidate neurophysiological markers of Alzheimer's disease
Vesna Jelic

CSF biomarkers in Alzheimer's disease, Parkinson's disease and atypical parkinsonism
Elka Stefanova

Neurodegenerative syndromes which manifest with parkinsonism and dementia
Milica Gregorič Kramberger

20:00—19:15 **Conference dinner: FENS - SiNAPSA social at the castle**

Sunday, 25 September

7:30—15:00 **Registration** | Second Foyer

8:30—10:00 Symposium | Kosovel Hall
Amygdala networks and the regulation of fear
Chair: Francesco Ferraguti

Structural and functional diversity of the intercalated cell masses of the amygdala and implications for fear learning
Francesco Ferraguti

Perisomatic inhibition in the basolateral amygdala and its control by inputs from basal forebrain
Norbert Hájos

Neuropetide S: control of state-dependent properties in the amygdala in instances of stress and fear
Hans Christian Pape

Neuropeptide Y modulates fear, anxiety and depression-like behavior in distinct nuclei of the amygdala
Ramon O. Tasan

8:30—10:00 Symposium | Linhart Hall
Lifetime development and reorganization of cortical circuitry
Chair: Ivica Kostović

Development of associative pathways in the human brain
Ivica Kostović

Molecular evolution and development of neural circuits of the cerebral cortex
Kyle Meyer

Extraordinary neoteny of the human prefrontal cortex: massive synaptic pruning on main projection neurons extends to third decade
Zdravko Petanjek

Association of cortical thickness and cognitive ability in children and adolescents
Sherif Karama

10:00—10:15 **Coffee break** | Second Foyer

10:15—11:45 Symposium | Kosovel Hall

The "grass roots" of plasticity in the brain: endocannabinoids as key regulators of synapses, networks and behaviors

Chair: István Katona

Molecular architecture of synaptic endocannabinoid signaling in the brain
István Katona

Astrocytes control spike-timing dependent plasticity at cortical synapses
Thomas Nevian

Dendritic and perisomatic inhibition in the hippocampal CA1 circuit
Attila Losonczy

Cannabinoid type 1 signaling: the "where" matters
Giovanni Marsicano

10:15—11:45 Symposium | Linhart Hall

Sex differences in brain and behaviour

Chair: Emilie Rissman

Sex chromosomes direct sex differences
Emilie Rissman

Sex differences in the brain: an interplay between genes and hormones
Gregor Majdič

Environment and brain sexual differentiation: what role for endocrine disrupters?
Giancarlo Panzica

Sexual differentiation of the human brain: consequences for gender-identity, sexual orientation and neuropsychiatric disorders
Dick Swaab

11:45—12:30 Plenary talk | Linhart Hall

Wired for sex: the neurobiology of Drosophila courtship behaviour
Barry J. Dickson

12:30—12:40 **Closing of the SiNAPSA Neuroscience Conference '11** | Linhart Hall

Poster sessions

Friday, 23 September

CEL-A01 **Unilateral entorhinal denervation leads to long-lasting dendritic alterations of mouse hippocampal granule cells**
Mario Vuksic

CEL-A02 **siRNA silencing of HIF-1α annulates protecive effect of hypoxia against induced apoptosis of human myoblasts exposed to 1% oxygen under in vitro conditions**
Katarina Pegan

CEL-A03 **Staurosporine induces apoptosis or primary necrosis in rat astrocytes**
Janez Šimenc

CEL-A04 **The effect of enriched environment breeding on the perineuronal nets and neurogenesis in tenascin C knockout mice**
Stefan Stamenkovic

CEL-A05 **Ischemia-induced neurogenesis in the long-term survival rat model**
Vera Sekeljic

CEL-A06 **Excitatory synaptic input controls the spiking activity of neurons during sharp wave-ripple oscillations in the CA3 region of hippocampal slices**
Rita Karlócai

CEL-A07 **Suppression of excitatory synaptic inputs onto CA3 pyramidal cells and fast spiking basket cells by CB1 cannabinoid receptor activation results in the impairment of hippocampal gamma oscillations**
Orsolya Papp

CEL-A08 **Effect of acute injection of fluoxetine in rats with constitutional upregulation/downregulation of platelet serotonin transporter**
Maja Kesic

CEL-A09 **Microcystin-LW induces apoptosis of rat cortical astrocytes**
Klara Bulc Rozman

CEL-A10 **Human anterior lens capsule epithelial cells contraction**
Sofija Andjelić

CEL-A11 **Characteristics of functioning of amygdalar neuronal network during unconditioned fear**
Maria P. Rysakova

CEL-A12 **Local synaptic connectivity in the adult auditory cortex**
Bruno M. Fontinha

CEL-A13 **Polyphenols can rescue neurons from necrotic and apoptotic cell death due to oxidative damage**
Lea Pogačnik

CEL-A14 **Alteration of brain circuits mediating fear and anxiety like behaviors in Steroidogenic factor 1 knockout mice**
Tomaž Büdefeld

CEL-A15 **Distribution and morphology of different GABAergic interneuron subpopulations in the human neocortex**
Domagoj Džaja

CEL-A16 **Abnormal regulation of the neuron-specific isoform of Elk-1 in response to l-dopa treatment in the 6-OHDA mouse model of Parkinson's disease**
Michael Feyder

CEL-A17 **Structural changes of GABAergic synapses upon fear conditioning in basolateral neurons of the mouse amygdala.**
Yu Kasugai

Saturday, 24 September

SYS-B06 **Early and late MRI changes in rat brain after prolonged seizures and nonspatial memory impairment**
Elena Suleymanova

SYS-B07 **Parvalbumin neurons and calretinin immunoreactive fibers degenerate in the subiculum after kainate-induced seizures in the rat**
Meinrad Drexel

SYS-B08 **Hypoxic preconditioning abolishes changes of CRH and vasopressin expression in hypothalamus triggered by inescapable stress in animal models of depression and anxiety**
Vera Mironova

SYS-B09 **Ingrowth of sensory axons into end-to-side neurorrhaphy – a retrograde tracer study in rat**
Tilen Žele

SYS-B10 **Neonatal exposure to organophosphorous substance chlormephos affect anxiety-like behaviour in adult mice, but does not permanently disrupt blood brain barrier**
Davor Ježek

SYS-B11 **The expression of cathepsin X and gamma enolase in mouse models of Alzeimer's disease and neuro inflammation induced by lipopolysaccharide**
Gordana Glavan

SYS-B12 **Comparative study of the influence of the acute administration of drugs of abuse on 50 kHz ultrasonic vocalization in male rats**
Nicola Simola

SYS-B13 **Aversive effects of ethanol. Ethanol-induced conditioned taste avoidance in male Wistar rats**
Beatriz González Segura

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Educational Workshop on Affective Neuroscience

Programme

www.sinapsa.org/snc11/workshop

Cankarjev dom, Ljubljana, Slovenia
24 September 2011

Saturday, 24 September

9:15—10:45 **Session I** | Linhart Hall

Affective cognitive neuroscience - an introduction to the workshop
Grega Repovš

Neurobiology of emotion
Luiz Pessoa

Emotional regulation and its breakdown in addiction
Hedy Kober

10:45—11:00 **Coffee break** | Second Foyer

11:00—12:30 **Session II** | Linhart Hall

Affective dysfunction in schizophrenia
Alan Anticevic

Role of emotion in delusion formation in schizophrenia
Philip R. Cortlett

Anomalous self-experience and affectivity in schizophrenia: a clinical-phenomenological approach
Borut Škodlar

Disintegration of emotional processing in dementias
Zvezdan Pirtošek

12:30—14:30 **Lunch break** | Second Foyer

14:30—16:00 **Session III** | Linhart Hall

Pain and emotion
Maja Bresjanac

Assessing emotion in a clinical setting
Jure Bon

Case report: Living without fear
Rok Berlot

Psychological pain and suicidal behavior – preliminary data
Peter Pregelj

Discussion and closing

16:00—16:15 **Coffee break** | Second Foyer

YNFL'11

Young Neuroscientist Forum Ljubljana '11
Faculty of Medicine University of Ljubljana
Ljubljana, Slovenia, September 22, 2011

Young Neuroscientists Forum Ljubljana 2011

Programme

www.sinapsa.org/snc11/ynfl11

Faculty of Medicine, Ljubljana, Slovenia
22 September 2011

Thursday, 22 September

7:30—9:00 **Registration and poster mounting**

9:00 **Opening of the Young Neuroscientists Forum Ljubljana '11**

9:00—10:45 **Student presentations**

YNFL-01 **Analysis of compound action potentials elicited in an insulated vagus nerve of a pig with selective vagus nerve stimulation**
Polona Pečlin

YNFL-02 **In vitro studies of EPM1 mutants of human stefin B**
Mira Polajnar

YNFL-03 **Investigating the "Tip-of-the-tongue" phenomenon**
Karmen Resnik

YNFL-04 **Changes at GABA-A receptors induced by long-term zolpidem treatment in primary culture of rat cerebellar granulle neurons**
Josipa Vlainić

YNFL-05 **The influence of nanosize titania on rat EEG power**
Anna Zelenskaya

YNFL-06 **A simple mathematical model accounts for the reactive electrocortical burst-suppression behavior during anesthetic coma**
Alexandra Oana Constantinescu

YNFL-07 **Brainstem tauopathy with progressive bulbar paralysis – a case presentation and analysis**
Nena Golob

YNFL-08 **Research for pathophysiology of early complications in acute spinal cord trauma**
Karolis Bareikis

YNFL-09 **Influence of Mozart's sonata K.448 on visual attention performance**
Simon Brezovar

YNFL-10 **Correlation between results of cognitive tasks performed with different emotions and EEG high frequency band**
Elena P. Krutenkova

YNFL-11 **An online brain-machine interface using decoding of movement direction from the human electrocorticogram**
Tomislav Milekovic

10:45—11:00 **Coffee break by the posters**

11:00—11:40 **Why should you give your life to neuroscience?**
Colin Blakemore

11:40—12:20 **Neuroscience and ethics**
Zvezdan Pirtošek

12:20—13:00 **Neuroscience and psychotherapy**
Maja Rus Makovec

13:00—14:00 **Lunch by the posters**

14:00—16:00 **Student presentations**

YNFL-12 **Morphological and quantitative analysis of neurons in lateral human hypothalamus and distribution of OX1R receptors**
Dimitrios G. Mytilinaios

YNFL-13 **Electroencephalographic and behavioural effects of intraperitoneal injection of grayanotoxin in adult Genetic Absence Epilepsy Rat from Strasbourg (GAERS)**
Pınar Kuru

YNFL-14 **Cognitive function in adults with type 1 diabetes mellitus**
Barbara Szémán

YNFL-15 **Multisensory integration in primary and supplementary visual cortices of the mouse: an in vivo 2-photon-calcium imaging study**
Anja Pahor

YNFL-16 **Neurofeedback training of the upper alpha frequency band in EEG improves cognitive performance**
Benedikt Zoefel

YNFL-17 **Phase of firing coding of sounds in the hippocampus**
Ekaterina Vinnik

YNFL-18 **Anatomical, neurochemical and functional consequences of selective cholinergic lesioning combined with local infusion of pre-aggregated amyloid peptide**
Giulio Kleiner

YNFL-19 **Noradrenergic contribution to spatial learning and memory: effects of selective lesion and tissue transplants**
Francesco Fieramosca

YNFL-20 **Neural stem cells-enriched tubulization improves anatomical and functional restoration of severed rat sciatic nerve**
Lucia Verga Falzacappa

YNFL-21 **An immunocytochemical tracer study of nigral dopamine neurons for the simultaneous double visualisation of tyrosine hydroxylase and fluorogold in light microscopy to investigate neuroprotection in a rat's model of Parkinson's disease following deep brain stimulation of the subthalamic nucleus**
Roxana Baclesanu

YNFL-22 **Activity of SKa-31 against seizure-like events in rat organotypic hippocampal slice cultures**
Muhammad Liaquat Raza

16:00 **Closing of the scientific programme of YNFL'11**

22:00 **Organised social programme in Ljubljana (Night sightseeing of Ljubljana's attractions and bars)**

International Course on EMG, SFEMG and Nerve Ultrasonography

Programme

www.sinapsa.org/snc11/sfemg

Cankarjev dom, Ljubljana, Slovenia
22—25 September 2011

Thursday, 22 September

14:00—20:00 **Registration** | Second Foyer

20:00—22:00 **Welcome reception: Wine and cheese by the posters** | Second Foyer
Co-hosted by SiNAPSA

Friday, 23 September

7:30—19:00 **Registration** | Second Foyer

9:15—9:25 **Welcome address and introduction to the course** | Štih Hall
Jože Trontelj

9:25—9:55 **Keynote Lecture: Ultrasonography of peripheral nerve & muscle in EMG lab** | Štih Hall
Luca Padua

9:55—10:20 **Theoretical principles of ultrasonographic diagnostics** | Štih Hall
Rok Hren

10:20—10:45 **Ultrasound anatomy and examination techniques in common compression neuropathies** | Štih Hall
Michaela Plaikner

10:45—11:00 **Cofee break** | Second Foyer

11:00—11:30 **Pathological changes in peripheral nerves** | Štih Hall
Roman Bošnjak

11:30—12:00 **Ultrasound guided neuromuscular interventions: nerve-instillations and biopsies** | Štih Hall
Alexander Loizides

12:00—12:30 **Setting-up of ultrasonographic activity – our experience** | Štih Hall
Simon Podnar

12:30—14:00 **Lunch break** | Second Foyer

14:00—14:30 **Ultrasound guided pain management in the cervical and lumbar spine** | Štih Hall
Alexander Loizides

14:30—15:00 **Ultrasound imaging of iatrogenic and other nerve lesions** | Štih Hall
Michaela Plaikner

15:00—16:00 **Demonstrations I** | Štih Hall, M3/4 Hall

16:00—16:15 **Cofee break** | Second Foyer

16:15—17:45 **Demonstrations II** | Štih Hall, M3/4 Hall

18:00—19:30 **Brainstorming clinical puzzles discussed with beer & snack** | Lili Novy Club

20:00—22:00 **Guided tour of the old town**

Saturday, 24 September

7:30—19:00 **Registration** | Second Foyer

9:15—9:45 **Introduction to single fiber EMG** | Štih Hall
Donald B. Sanders

9:45—10:15 **Advanced neurography & late responses: update** | Štih Hall
Erik Stålberg

10:15—10:45 **Stimulation SFEMG: the need for good technique** | Štih Hall
Jože Trontelj

10:45—11:00 **Cofee break** | Second Foyer

11:00—11:30 **Keynote Lecture: The contribution of electromyography to the understanding of changes in myopathic muscle** | Štih Hall
Erik Stålberg

11:30—12:00 **Stimulated single fiber EMG at different frequencies in human botulism** | Štih Hall
José M. Fernández

12:00—12:30 **Computer program for the electrodiagnostic evaluation of patients with suspected carpal tunnel syndrome** | Štih Hall
Simon Podnar

12:30—14:00 **Lunch break** | Second Foyer

14:00—16:00 **Demonstrations III** | Štih Hall, M3/4 Hall

16:15—17:00 **Demonstrations IV** | Štih Hall, M3/4 Hall

17:00—17:45 Dr. Janez Faganel Memorial Lecture | Linhart Hall
Biomarkers for myasthenia gravis
Donald B. Sanders

20:00—22:00 **Conference dinner: FENS - SiNAPSA social at the castle**

Sunday, 25 September

7:30—15:00 **Registration** | Second Foyer

8:30—10:00 **Short oral presentations** | Štih Hall

An unusual presentation of Lambert-Eaton myasthenic syndrome
Siti Aisyah Yaacob

Lambert-Eaton myasthenic syndrome – repetitive stimulation in 3 patients
Eduard Minks

Effect of bipolar electrostimulation on focal neuropathy of ulnar nerve in elbow area – pilot study
Ivica Husárová

Inching in focal neuropathy of the ulnar nerve in the elbow
Tomas Gescheidt

Median nerve F-wave analysis in patients with carpal tunnel syndrome
Jose M. Fernandez

10:00—10:15 **Cofee break** | Second Foyer

10:15—10:35 **Results to be expected with stimulation SFEMG in early myasthenia gravis** | Štih Hall
Jože Trontelj

10:35—10:55 **Stimulation SFEMG in studies of neuromuscular physiology** | Štih Hall
Jože Trontelj

10:55—11:45 **Demonstrations V** | Štih Hall, M3/4 Hall

			Štih	M3/4-A	M3/4-B	M3/4-C	M3/4-D
Demo I	Friday, 23. 9.	15:00-16:00	6 LP	8 AL/MP	2 JT	3 ES	1 DS
Demo II	Friday, 23. 9.	16:15-17:45	7 LP	6 AL/MP	2 JT	5 JZ	4 DS
Demo III	Saturday, 24. 9.	14:00-16:00	3 ES	6 MP	8 AL	4 DS	1 JF
Demo IV	Saturday, 24. 9.	16:15-17:00	1 DS	7 MP	6 AL	3 ES	2 JF
Demo V	Sunday, 25. 9.	10:55-11:45	2 JT	3 DS	5 SP	1 ES	4 JF

1 Voluntary SFEMG, fibre density, jitter, facial and limb muscles

2 Stimulation SFEMG, jitter, facial and limb muscles

3 Jitter with concentric EMG electrode, with voluntary activation and axonal stimulation

4 Repetitive nerve stimulation

5 Phrenic nerve conduction and diaphragm needle EMG

6 Basic peripheral nerve and musculo-skeletal ultrasnography of nerves and muscles

7 Advanced peripheral nerve ultrasound: border nerves, lateral femoral cutaneous nerve, and others

8 Advanced peripheral nerve ultrasound: the spine and US-guided interventions

AL Alexander Loizides

DS Donald B. Sanders

ES Erik Stålberg

JF Jose Fernandez

JT Jože Trontelj

JZ Janez Zidar

LP Luca Padua

MP Michaela Plaikner

SP Simon Podnar

SNC'11
SiNAPSA Neuroscience Conference '11
Central European FENS Featured Regional Meeting
Ljubljana, Slovenia, September 22-25, 2011

Clinical Satellite Symposia

(in Slovene)

www.sinapsa.org/snc11/simpoziji

Cankarjev dom, Ljubljana, Slovenia
23—24 September 2011

Kako uspešno odkrivamo in zdravimo demenco?

Srečanje, ki bo potekalo 23. septembra 2011 v Kosovelovi dvorani Cankarjevega doma s pričetkom ob 17. uri, organizira Krka v sodelovanju z Združenjem psihiatrov in Združenjem nevrologov SZD.

moderatorja:	Peter Pregelj, Anton Mesec
17:00–17:30	Demence včeraj, danes, jutri Aleš Kogoj, Zvezdan Pirtošek
17:30–17:50	Obravnava bolnikov z demenco: pogled psihiatra Aleš Kogoj
17:50–18:10	Obravnava bolnikov z demenco: pogled nevrologa Maja Trošt
18:10–18:30	Vloga družinskega zdravnika v obravnavi bolnika z demenco Marko Drešček
18:30–18:50	Odmor
18:50–19:10	Psihiatrični simptomi pri demenci Peter Pregelj
19:10–19:30	Na terapevtskem obzorju Gorazd B. Stokin
19:30–19:40	Krka v zdravljenju demence
19:40–19:50	Diskusija
20:00–22:00	Sprejem v Klubu CD

Nevroplastičnost pri duševnih motnjah

Srečanje, ki bo potekalo 24. septembra 2011 v dvorani M1 Cankarjevega doma s pričetkom ob 15:30, organizira Eli Lilly v sodelovanju z Združenjem psihiatrov in Združenjem nevrologov SZD.

15:30–16:00	Nevroplastičnost pri duševnih motnjah Jurij Bon

Rezistentne epilepsije

Srečanje, ki bo potekalo 24. septembra 2011 v Klubu Cankarjevega doma s pričetkom ob 16:15, organizira GlaxoSmithKline v sodelovanju z Združenjem psihiatrov in Združenjem nevrologov SZD.

16:15–16:35	Aktualna problematika obravnave epilepsij - lokalen pogled na globalen problem Igor M. Ravnik
16:35–17:00	Možnosti v zdravljenju rezistentnih epilepsij Bogdan Lorber

SNC'11

SiNAPSA Neuroscience Conference '11

Central European FENS Featured Regional Meeting

Ljubljana, Slovenia, September 22-25, 2011

General Information

www.sinapsa.org/snc11

Cankarjev dom, Ljubljana, Slovenia
22—25 September 2011

GENERAL INFORMATION

Venue

Cankarjev dom, Cultural and Congress Centre

Prešernova 10, SI-1000 Ljubljana, Slovenia

Phone: +386 1 241 7100

Fax: +386 1 241 7296

Information for Poster Presenters

The required poster dimensions are 190 cm (width) by 100 cm (height), and will be mounted in landscape orientation (poster board size 200 cm x 120 cm).

Due to large number of posters, each poster will be exhibited for two days only. For that purpose, posters will be assigned to either a group A or B. For group A, the posters must be mounted on Thursday before 16:00 and removed by 19:00 hrs on Friday. For Group B, the posters should be mounted on Saturday between 8:00 and 9:00 hrs and removed by the end of scientific programme on Sunday.

Authors are requested to be by their posters at the specified time for presentations, i.e., between 12:30 and 14:30 hrs. In addition, we advise that they stay in the poster area also during coffee breaks for informal discussion of their work.

Information for Speakers

A Speaker Centre with technical equipment and staff to review media and download your presentation will be available, and a technician and a room attendant will be in every room to provide assistance when needed. The Speakers Centre will have the same opening hours as the registration desk, i.e. it will open at 2 p.m. on Thursday 22 September, and at 7:30 a.m. on every subsequent day of the conference.

Please make sure that your presentation is installed and tested at least two hours before your talk. Only Power Point presentations, CDROM, USB Memory cards will be accepted for downloads on the lecture hall computer. Version MS PowerPoint 2007 is recommended.

At the end of the congress, all presentations will be deleted from the lecture hall and speaker center computers.

Business Centre

The following services are available in the Business Centre in M2 Hall: internet terminals, e-mail and word processors, while internet connection is also possible when using your own notebook.

Internet

Wireless internet connection is available in Second Foyer.

Conference Identification Badge

A conference identification badge will be included in the conference material provided upon registration. There will be no admittance to the Scientific Sessions without the conference badge. Invitations to social events will be collected at the entrance.

Attendance Certificate

A Certificate of Attendance will be issued to all registered participants.

CME Certificate

Continuous Medical Education (CME) certificates will be issued to registered members of the Slovenian and the Croatian Medical Chambers.

Coffee Breaks

During breaks, refreshments will be served free of charge to participants wearing congress badges.

Lunches

Lunch boxes will be available for purchase on site during lunch time/poster sessions on Friday and on Saturday.

COMMITTEES AND ORGANISATION

SNC'11 Programme Committee

Grega Repovš (Chair)
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Lea Leonardis

REGISTRATION AND FEES

Registration and Information Desk

The Registration Desk located in Second Foyer of Cankarjev dom will open as follows:

Thursday, 22 September 14:00–20:00
Friday, 23 September 7:30–19:00
Saturday, 24 September 7:30–19:00
Sunday, 25 September 7:30–15:00

Registration fees

Full registration includes:

- Admission to all SNC'11 lectures and access to the poster/exhibition area
- Participation in Educational Workshop on Affective Neuroscience on Saturday
- Congress materials including Book of Abstracts
- Wine and Cheese Welcome Reception in the poster area on Thursday
- Coffee in the poster/exhibition area during breaks
- Ljubljana Sightseeing on Friday
- Conference Dinner at the Castle on Saturday

Daily registration includes:

- Admittance to all SNC'11 lectures and access to the poster/exhibition area on the selected day
- Participation in Educational Workshop on Affective Neuroscience – Saturday daily registration
- Coffee in the poster/exhibition area during breaks on the selected day

Graduate student and clinical trainee registration includes:

- Admittance to all SNC'11 lectures and access to the poster/exhibition area
- Participation in Educational Workshop on Affective Neuroscience on Saturday
- Congress materials including Book of Abstracts
- Wine and Cheese Welcome Reception in the poster area on Thursday
- Coffee in the poster/exhibition area during breaks
- Ljubljana Sightseeing on Friday
- Conference Dinner at the Castle on Saturday

Undergraduate student registration includes:

- Participation in Young Neuroscientists Forum (22 September)
- Admittance to all SNC'11 lectures and access to the poster/exhibition area
- Participation in Educational Workshop on Affective Neuroscience on Saturday
- Book of Abstracts
- Wine and Cheese Welcome Reception on Thursday
- Coffee in the poster/exhibition area during breaks
- Ljubljana Sightseeing on Friday

Course on EMG, SFEMG and Nerve Ultrasonography registration includes:

- Participation in EMG & US Course workshops and lectures
- Admittance to all SNC'11 lectures and access to the poster/exhibition area
- Congress materials including Book of Abstracts
- Wine and Cheese Welcome Reception on Thursday
- Coffee in the poster/exhibition area during breaks
- Ljubljana Sightseeing on Friday
- Conference Dinner at the Castle on Saturday

SOCIAL AND TOURIST PROGRAMME

Welcome Reception – Wine & Cheese by the Posters

Thursday, 22September, 20:00–22:00

Wine & Cheese will be offered by the posters (Second Foyer) on Thursday evening to welcome all the participants to the conference and offer the first opportunity to browse through the poster and exhibitor area and mark points of special interest for the remaining days at the SNC'11.

Sightseeing Tour of Ljubljana

Friday, 23 September, 20:00–21:00

The tour includes a walk past the major sights of the old part of Ljubljana. The old city centre has a unique architectural appearance, particularly due to its mixture of Baroque and Art Nouveau architecture with masterful creations by the 20th century architect Jože Plečnik.

Meeting point: 20:00 at Prešeren Square

Conference Dinner – FENS-SiNAPSA Social at the Castle

Saturday, 24 September, 20:00–23:00

FENS and SiNAPSA invite the regular participants and those who obtain the tickets to the Conference Dinner and Social at the Ljubljana Castle on Saturday evening.

Buses will depart from Cankarjev dom (exit onto Erjavčeva street) at 19:30.

Recommended attire - elegant casual.

Included in the fee for participants (except one-day & undergraduate registration). Additional tickets: EUR 40 / person

Excursion to the Postojna Cave and Predjama Castle

Saturday, 24 September, 9:00–15:00

Meeting point: Registration desk in Cankarjev dom.

Dress: Please note that the temperature in the caves is approximately 8 degrees Celsius. Bring warm clothing and comfortable shoes!

This half day excursion will take you to the biggest and worldwide known Slovenian cave system - the Postojna Cave. A two million years old and 27 km long system of subterranean caves is one of the most easily accessible networks of underground caves in the world. A ride by electrical train will be followed by a guided walk through a series of cave halls with drop stones, pillars and translucent curtains that create unforgettable impressions. The underworld system is a home of the mysterious, unique and rare amphibian Proteus Anguinus, or "human fish" as it is popularly called here.

Only 9 km away stands one of the most interesting baroque castles of Slovenia, the Predjama Castle. It hangs dramatically in the middle of 123 m high rocky cliff.

Price: 65 EUR per person (min 15 persons)

Included: bus transfer, English-speaking guide, entrance fee to Postojna Cave, entrance fee to Predjama Castle

Excursion to Bled

Sunday, 25 September, 14:00–19:00

Meeting point: SNC'11 Registration desk

Often described as the 'Image of Paradise', the Alpine resort town of Bled will enchant you with its emerald-green lake, fairytale-like island and imposing castle reigning on top of a rocky cliff. This harmonious mix of beautiful nature, rich history and genuine hospitality has been attracting visitors for centuries. It was here that the Yugoslavia Royal Family once had a summer residence, later replaced by the summer residence of President Tito, which can still be seen by the lake.

After an enjoyable 56 km drive from Ljubljana, we board a traditional 'pletna' boat and smoothly glide over the lake to visit the only Slovenian island. Its top is crowned with a little church, and you will have an opportunity to ring the mysterious wishing bell. A scenic road takes us around the lake and to the medieval castle perched high on a cliff. Built over a thousand years ago, it was once an unconquerable property of the Bishops of Brixen. Today, it attracts visitors with its museum, and demonstrations of old crafts and traditions, not to mention the breathtaking view of the surroundings.

Price: 68 EUR per person (min 15 persons)

Included: bus transfer, English-speaking guide, ride with 'pletna', entrance fee to the church on the isle, entrance fee to Bled Castle

ACKNOWLEDGEMENTS

The organizers thank the following organizations for supporting the SiNAPSA Neuroscience Conference '11, a Central European FENS Featured Regional Meeting:

FENS, Federation of European Neuroscience Societies

IBRO, International Brain Research Organization

EC-IFCN, European Chapter of the International Federation of Clinical Neurophysiology

ARRS, Slovenian Research Agency

Faculty of Medicine, University of Ljubljana

University Medical Centre Ljubljana

SiNAPSA Neuroscience Conference '11 is organized under the auspices of the Slovenian Academy of Sciences and Arts.

SNC'11

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SNC'11 Plenary and Special Talks

Abstracts

www.sinapsa.org/snc11

Cankarjev dom, Ljubljana, Slovenia
22—25 September 2011

Thursday, 22 September, 16:00

Andrej O. Župančič Memorial Lecture

The α,β-hydrolase fold: offering adhesion and catalysis within the synapse

Palmer Taylor

Department of Pharmacology, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego

Often in the neurosciences one gains additional insights into structure and function by comparing parallels in signaling pathways, electrophysiologic events, molecular interactions and atomic structures. This Zupancic Lecture will attempt to examine such relationships in two arenas. The first relates to recognition properties of molecules involved in neurotransmission. In the cholinergic nervous system, we find proteins engaged in the biosynthesis of acetylcholine; the transport of an immediate precursor, choline, and acetylcholine itself; its interaction with distinctive receptor systems (nicotinic and muscarinic) and its catalysis to terminate neurotransmitter action, all being handled by distinctive molecules with no real homology or amino acid identity in any of their domains. Yet, if the common property for recognition for acetylcholine were extended to other molecules that interact with each of these proteins, evolution that is dependent on predation and protection from predation and the very discipline of pharmacology as a science would lack discriminatory power. With structures of each of the above proteins mediating neurotransmission now succumbing to atomic resolution technologies, we can ascertain how nature has developed molecules to recognize a common transmitter, yet have disparate recognition capacities for its close congeners. In particular, we will consider acetylcholinesterase and the nicotinic receptor in this comparison.

A second arena for comparison involves structure, biosynthesis and function of proteins with sequence homology. Apart from the expectation of finding other esterases related to cholinesterase in a common α,β-hydrolase-fold family, we find proteins of diverse functions falling into this protein superfamily with a common structural fold. The tactin family in which neuroligin is the central representative in mammalian systems possesses adhesive properties with no catalytic function. Neuroligin is a trans-synaptic adhesion protein located post-synaptically, whose only known partners are the α and β-neurexins on the pre-synaptic surface. Accordingly, cholinesterase and neuroligin are two synaptic proteins with disparate functions, but possessing a common fold dictated by homologous sequence. A third function is found in another homologous protein, thyroglobulin, the precurser to thyroid hormone, where the cholinesterase homologous domain subserves a chaperone role in promoting secretion and storage of a larger protein domain that is processed to form the iodinated hormones. Similarily, cholinesterase itself could serve as its own intrinsic chaperone serving to traffic the structural subunits with which it associates in the biosynthetic process. What is also common among these α,β-hydrolase fold proteins are mutations that give rise to folding deficiencies. Common mutations in these proteins give rise to similar aberrations in folding therein producing deficiences in hydrolysis of neurotransmitters and drugs, thyroid hormone deficiencies, and deficits in neuronal development reflected in autism spectrum disorders and other disorders of development in the nervous system. Hence, in this second example, despite very disparate functions of the α,β-hydrolase fold proteins, their structures reveal commonalities in protein biosynthesis and trafficking and aberrations of these processes observed in disease states.

Thursday, 22 September, 17:30

Principles and mechanisms of neuronal migration: relevance for human brain disorders

Pasko Rakic

Department of Neurobiology and Kavli Institute for Neuroscience at Yale, New Haven, USA

The identity, synaptic relationship and, ultimately, function of neurons is defined by their position. This is particularly evident in the cerebral cortex where neurons acquire their proper areal, laminar and columnar position during development by active migration from multiple sites of origin and involve complex molecular events and cell-cell interactions. I will describe how specific genes and variety of morphoregulatory and signaling molecules cooperate in orchestrating various components of neuronal migration. We found that the rate of neuronal migration can be disturbed by manipulation of selected gens or exposure of embryo to various physical, chemical, and biological agents. Consequences of disruption or even slowing down of neuronal migration can range from gross heterotopias to subtle abnormalities of neuronal positions that eventually affect the pattern of synaptic circuits and may ultimately cause variety of neuropsychiatric disorders.

Thursday, 22 September, 18:15

Neuroscience and Society special event

Ethical dilemmas in neuroscience

Colin Blakemore, Jože Trontelj, Marian Joels, Gilberto Pizzolato, Srećko Gajović, Luka Omladič

Panel Discussion

Through their brief presentations and a moderated panel discussion, a group of scientists will tackle some of the ethical dilemmas facing neuroscientists and society today, and answer select questions from the audience.

Our brains are responsible for all our thoughts and actions. The ethics of neuroscience research is, then, philosophically intriguing. Is the organ of ethics capable of explaining its own ethical judgements and behaviour? Beyond this little philosophical paradox, it is clear that research on the brain, and its applications in everyday life, raise important ethical questions.

Is it acceptable to use animals in research, especially when that research is directed at understanding diseases or processes (such as pain) that are likely to cause suffering?

Should we set limits to the use of neuroscientific techniques (especially neuroimaging) to monitor personal, subjective experiences and intentions, and the use of methods of brain stimulation to methods of modulating and modifying brain activity and behaviour?

How should we approach the potential military use of neuroscientific knowledge (e.g. the possible development of new methods of incapacitating or altering the thought processes of populations?

Do we need to reconsider the basis of our legal systems as our understanding of the causal basis of decision-making casts doubt on the standard concepts of free will and responsibility?

Consideration of ethical issues arising from advances in science should not be left to scientists alone: the general public and politicians must be involved in making difficult ethical decisions. But scientists themselves should lead the debate. Neuroscientists have a responsibility to inform the public about the ethical issues raised by their research, and to provide the information and evidence on which sensible decisions can be made.

Friday, 23 September, 08:30

Neural plasticity: liberating the brain from its genetic constraints

Colin Blakemore

Department of Physiology, Anatomy and Genetics, University of Oxford, UK

Development of complex neural circuits depends on general rules, specified by genetic instructions, with adaptive mechanisms to fine-tune connectivity. Such flexibililty, essential for development, might have underpinned evolutionary changes in complex brains. In addition, synaptic plasticity enables neurons to change the strength of their connections in response to the pattern of activity passing through them, helping individuals to match perceptual, cognitive and motor skills to the nature of the world around them. Neuronal plasticity, although genetically determined, enabled humans to escape from the informational limits in the blueprint of their genes and propelled them into a different mode of evolution.

Keywords: plasticity, evolution, cerebral cortex, development

Friday, 23 September, 16:15

On the relationship between emotion and cognition

Luiz Pessoa

Department of Psychology, University of Maryland, College Park, USA

The current view of brain organization supports the notion that there is a considerable degree of functional specialization and that many regions can be conceptualized as either 'affective' or 'cognitive'. Popular examples are the amygdala in the domain of emotion and the lateral prefrontal cortex in the case of cognition. This prevalent view is problematic for a number of reasons. It will be argued that complex cognitive–emotional behaviors have their basis in networks of brain areas, none of which should be conceptualized as specifically affective or cognitive. Central to cognitive–emotional interactions are brain areas with a high degree of connectivity called hubs (e.g., amygdala), which are critical for regulating the flow and integration of information between regions. To illustrate cognitive-emotional processing, I will discuss a series of studies that have investigated interactions between emotion and perception (including studies showing that emotional perception is not automatic) and, more recently, between emotion and executive function.

Friday, 23 September, 17:00

EJN Best Publication Award 2011

A common molecular basis for membrane docking and functional priming of synaptic vesicles

L. Siksou, F. Varoqueaux, O. Pascual, A. Triller, N. Brose, S. Marty

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Synaptic vesicle excoytosis is thought to be preceded by docking and priming of synaptic vesicles to the presynaptic plasma membrane. Docking is defined morphologically, as the capacity of synaptic vesicles to anchor to the plasma membrane, and priming, electrophysiologically, as the ability for synaptic vesicles to be readily released. Synapses from Munc13 proteins deficient mice are unable to release synaptic vesicles and show no defect in docking in electron microscopy studies using aldehyde-fixation. However the number of docked synaptic vesicles is reduced in studies using high-pressure freezing (a technique that circumvents aldehyde artifacts and allows fast immobilization of the tissue) in C. elegans Unc13 mutants. We reevaluated synaptic vesicles docking in mice lacking Munc13 proteins using high-pressure freezing and electron tomography. In controls, docked synaptic vesicles are in contact with the plasma membrane and linked to presynaptic dense material by filaments. However, in Munc13 deficient mice, synaptic vesicles remain at a distance from the plasma membrane. Munc13 proteins are thus required to dock synaptic vesicles at the plasma membrane on top of their role in vesicle priming. These results argue for common molecular mechanisms sustaining synaptic vesicles priming and docking processes.

European Journal of Neuroscience, 30(1): 49–56 (2009).

Friday, 23 September, 18:00

Meet FENS

Sten Grillner, Fotini Stylianopoulou, Ole Kiehn, Jean-Marc Fritchy

Federation of European Neuroscience Societies

FENS would like to inform you about the FENS mission and the current initiatives to promote Neuroscience, at the European, national and individual laboratory level, and the FENS programmes that may boost neuroscience education and research and the need for promotion of neuroscience from the funding perspective.

The official journal of FENS, European Journal of Neuroscience is a most visible medium for publishing your work and accessing high quality publications of others in the field.

Saturday, 24 September, 08:30

Classifying clinical images: a new aid to dementia diagnosis with implications for treatment strategies

Richard Frackowiak

Department of Clinical Neuroscience, Service of Neurology, CHUV University Hospital, Lausanne, Switzerland

The application of modern computerized automated techniques for analyzing structural and functional brain images have led to real advances in the application of advanced neuroimaging to clinical practice in ways undreamed of only a short time ago. One major advance is the development of image classification techniques that puts diagnosis of the individual at the centre of the enterprise. This approach is currently based on machine learning techniques, some of the best results being obtained with support vector machines (SVM). There is a lot of activity in this area at present and new methods of analysis and results are constantly being reported. MR scanner manufacturers are becoming interested in translating these encouraging results into potential products.

Thus, neuroimaging techniques, in addition to their traditional diagnostic role are currently expanding understanding of the structural and functional changes that occur in dementia. Further research may allow identification of early pathological signs of AD, before clinical symptoms are evident, providing the opportunity to test preventative therapies.

The lecture will review imaging in Alzheimer's disease and other neurodegenerative diseases and attempt to project into the future how the field will develop. Additionally obstacles to such developments will be highlighted and approaches to validating image classification as a diagnostic tool and a means of monitoring treatment discussed.

Keywords: machine learning, neurodegeneration, neuroimaging, support vector machines

Saturday, 24 September, 16:15

Untangling the role of protein aggregation in neurodegenerative diseases

Maria Grazia Spillantini

Cambridge Centre for Brain Repair, Department of Clinical Neurosciences, University of Cambridge, UK

Parkinson's disease (PD) is the most common movement disorder. Clinically is characterized by rigidity, resting tremore and bradikinesia which are associated with degeneration of neurones in the substantia nigra and other brain regions. Although the movement disorder is the main feature of (PD), non motor symptoms such as depression, disturbed sleep and intestinal problems can precede the motor dysfunction. Neuropathologically PD is characterized by the presence of intracellular filamentous protein aggregates known as Lewy bodies and Lewy neurites in neurones of the substantia nigra and other brain regions. Indeed Braak et al. have shown that Lewy bodies are present very early in in the gut of PD patients and concluded that the pathology progresses from the gut to the brain through neuronal networks. Genetic mutations and multiplications of the alpha-synuclein gene have been found to be the cause of familial forms of PD and alpha-synuclein has been also shown to be the major componenet of the Lewy bodies. These two findings clearly associate alpha-synuclein to the pathogenesis of the PD but the contributions of Lewy bodies to neurodegeneration and the mechanism leading to their formation remain unclear. We have produced a transgenic mouse models that expresses truncated human alpha-synuclein under the control of the tyrosine hydroxilase promoter in dopaminergic neurons. In these mice, both in the presence or absence of endogenous alpha-synuclein, truncated alpha-synuclein aggregates into granular and filamentous material and this aggregation is associated with progressive reduction in dopamine release, dopamine loss and appearance of motor impairment. The reduction of dopamine release is associated with alpha-synuclein aggregates at the presynaptic terminal and redistribution of the SNARE complex involved in neurotransmitter release. In this mouse model we find some accumulation of alpha-synuclein also in the gut. These mice represent a good model where to investigate the mechanisms associated to alpha-synuclein aggregation.

Saturday, 24 September, 17:00

Dr. Janez Faganel Memorial Lecture

Biomarkers for myasthenia gravis

Donald B. Sanders

Duke University Medical Center, Durham, USA

A biological marker or biomarker is an objectively measured characteristic that indicates a normal or pathogenic biological process, or reflects a biological response to a therapeutic intervention. Biomarkers are essential in clinical trials to measure disease severity or the therapeutic activity of a treatment under investigation. In myasthenia gravis (MG), potential biomarkers include serum antibodies and neuromuscular jitter.

To assess and compare the potential value of these biomarkers in MG, we reviewed their correlation with disease severity and responsiveness to clinical change in a large population of patients.

Serum antibodies to the acetylcholine receptor or MuSK are found in more than 90% of MG patients, and generally fall with immunosuppressive treatment or after thymectomy. However, the change may be a non-specific response to immunosuppression and frequently does not correlate with clinical change, limiting their role as biomarkers.

Three parameters of jitter measurement (the mean MCD, the percent of pairs with blocking, and the percent of pairs with normal jitter) in the EDC and frontalis each correlate with overall disease severity; measurements in the frontalis discriminate better between remission and ocular disease, and would be preferred as a marker of disease severity.

Calculation of composite Z-scores for these parameters demonstrated that a formula using mean MCD and % normal endplates best reflects change in disease severity.

It is concluded that measurement of jitter is the best pharmacodynamic biomarker for monitoring disease activity in MG. Because the requisite expertise is not widely available, which limits its use in clinical trials, jitter measurement would be most useful in early phase studies.

Sunday, 25 September, 11:45

Wired for sex: the neurobiology of Drosophila courtship behaviour

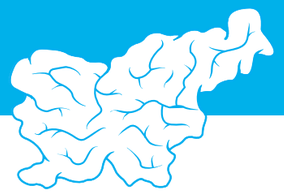
Anna von Philipsborn, Krystyna Keleman, Jai Yu, Tianxiao Liu, Barry J. Dickson

Research Institute of Molecular Pathology, Vienna, Austria

How are innate behavioural repertoires pre-programmed into the nervous system? And how does trial-and-error learning allow each individual to fine tune this innate template to adapt to the conditions of the local environment? The courtship behaviour of Drosophila melanogaster males is an ideal model system to address these questions at the level of single cells and genetically-defined neural circuits.

I will present our current understanding of the anatomy and function of neural circuity that generates male courtship behaviour. This analysis has revealed how sexual dimorphisms sculpted into these circuits by the fruitless gene shape the distinct behaviours of males and females. We have also uncovered elements of this circuit that mediate dopamine-dependent learning in the adult fly, so that his courtship activity is preferentially directed at receptive virgin females. These studies are beginning to reveal the cellular and circuit mechanisms underlying innate and learned behaviours in this model system.

Keywords: behaviour, neural circuit, Drosophila, courtship, dopamine, learning



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Cankarjev dom, Ljubljana, Slovenia
22—25 September 2011

The role of changes in the GABA system and of endocanabinoids in epileptogenesis

Zsofia Magloczky

Institute of Experimental Medicine, Hungarian Acadademy of Sciences, Budapest, Hungary

Inhibition is a crucial mechanisms in epilepsy. Beside the interneuronal network other factors also contribute in the regulation of GABAergic synaptic transmission. The endocannabinoid system plays a central role in retrograde synaptic communication, and controls both glutamatergic and GABAergic transmission via type 1 cannabinoid receptors (CB1). Both in sclerotic human hippocampi and in the chronic phase of pilocarpine-induced epilepsy in mice with sclerosis, CB1 receptor-positive interneuron somata were preserved both in the dentate gyrus and in the CA1 area, and the density of CB1 immunostained fibers increased considerably in the dentate molecular layer. This suggests that, while CB1 receptors are known to be reduced in density on glutamatergic axons, the CB1 receptor-expressing GABAergic axons sprout, and/or there is an increase of CB1 receptor levels on these fibers. The changes of CB1 immunostaining in association with the GABAergic inhibitory system appears to correlate with the severity of pyramidal cell loss in the CA1 subfield. These results confirm the involvement of the endocannabinoid system associated with GABAergic transmission in human TLE, as well as in the chronic phase of the pilocarpine model in mice. Pharmacotherapy aimed at the modulation of endocannabinoid-signaling should take into account the opposite change in CB1 receptor expression observed on glutamatergic versus GABAergic axon terminals.

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The role of neurogenesis in the development of epilepsy

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Seizures increase neurogenesis in subgranular zones of the dentate gyrus of the hippocampus, which is one the most neurogenic regions of the adult rodents and apparently human brain. However, the role of increased neurogenesis in the hippocampus in ictogenesis and epileptogenesis remains elusive. Therefore, it is important to investigate how the cells that are born in response to epileptic seizures are integrated into the existing neuronal networks in terms of their functional contribution to the network activity. Such studies would shed light on how such functional integration contributes to the excitability of the hippocampal network. This will in turn determine whether increased neurogenesis is beneficial or detrimental against ictogenesis and epileptogenesis. Some of the crucial factors affecting the functional integration of newborn cells seems to be excessive neuronal activity and/or inflammatory microenvironment, both associated with acute, as well as chronic epileptic conditions. Our data suggest that these aspects determine the pattern of functional integration of newborn cells in animal models of epilepsy with various degrees of seizure severity and associated microenvironmental alterations in the brain tissue.

A role for leukocyte-endothelial adhesion mechanisms in epilepsy

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Epilepsy is a neurological disorder affecting 0.5-1% of the general world population. Experimental and clinical data indicate that inflammatory condition can mediate neurodegenerative mechanisms. It becomes thus imperative to investigate such mechanisms in order to identify any possible pharmacological treatment to prevent that single episode of brain insult can lead to a condition of epilepsy. We recently reported in pilocarpine-induced epilepsy in C57Bl/6 mice that seizures upregulate expression of vascular cell adhesion molecule-1 (VCAM-1) and selectins, as well as an enhanced granulocyte and Th1 lymphocyte rolling and arrest in brain vessels. Furthermore, we demonstrated that inhibition of leukocyte adhesion mechanisms leads to a significant reduction of disease severity. To further validate the role of leukocyte adhesion mechanisms in the pathogenesis of epilepsy we performed in vivo staining to study the expression of mucin and integrin ligands in brain venules after status epilepticus induced by kainic acid (KA). Our results support previous data obtained in pilocarpine model and show that KA-induced seizures induce expression of VCAM-1, ICAM (intercellular adhesion molecule)-1 and P-selectin shortly after seizures, suggesting that seizure activity per se activates brain endothelium potentially allowing blood leukocyte adhesion and endothelial damage. We next asked whether the inciting molecule pilocarpine is able to directly activate mouse brain endothelium in vitro. We cultured mouse brain endothelial cells in the presence of pilocarpine for different time periods and then performed flow cytometry to study the expression of endothelial adhesion molecules. Our data show that pilocarpine rapidly upregulates P-selectin, the main ligand for mucin PSGL (P-selectin glycoprotein ligand)-1, an adhesion molecule controlling leukocyte rolling. In addition, we observed upregulation of VCAM-1 and ICAM-1 expression after treatment with pilocarpine, suggesting that brain endothelium upregulates also ligands for beta1 and beta 2 integrins, molecules implicated in leukocyte migration through the endothelial lining. Moreover, preliminary results obtained using Bioplex technology suggest that brain endothelium produces cytokines after stimulation with pilocarpine in vivo and in vitro. In conclusion, our results further support a role for inflammation mechanisms in the pathogenesis of epilepsy induced by pilocarpine.

Over-expression of GABA system in the dentate gyrus of patients with temporal lobe epilepsy patients indicates a protective mechanism

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Recently, expression of glutamate decarboxylase-67 (GAD67), a key enzyme of GABA synthesis, was detected in the otherwise glutamatergic mossy fibers of the rat hippocampus. Synthesis of the enzyme was markedly enhanced after experimentally induced status epilepticus. Here, we investigated the expression of GAD67 protein and mRNA in hippocampal specimens from 44 patients with mesial temporal lobe epilepsy (TLE) using double immunofluorescence histochemistry, immunoblotting and in situ hybridization. GAD67 was highly expressed in terminal areas of mossy fibers, including the dentate hilus and the stratum lucidum of sector CA3. In cases with Ammon's horn sclerosis, the inner molecular layer of the dentate gyrus where sprouted mossy fibers terminate contained also high levels of GAD67 immunoreactivity. Double immunofluorescence revealed the co-localization of GAD67 immunoreactivity with the mossy fiber marker dynorphin. Furthermore, GAD67 mRNA was found in granule cells of the dentate gyrus. Levels, both of GAD67 mRNA and of GAD67 immunoreactivity were increased in the TLE specimens compared with post mortem controls. Our data indicate expression of GAD67 and therefore synthesis of GABA in hippocampal mossy fibers of TLE patients. This may represent a self-protecting mechanism in TLE. GABA released from mossy fibers may contribute to an amelioration or termination of seizure activity.

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Short and long term structural plasticity in the mouse neocortex

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Experience-dependent cortical plasticity is thought to be an important aspect of perceptual learning, and likely depends on activity-dependent strengthening and weakening of pre-established synapses as well as on structural plasticity, including synapse formation and elimination. We perform long-term and short-term time-lapse imaging of GFP-expressing neurons in the mouse barrel cortex in vivo. We detect rapid and slow structural modifications of synaptic connections after LTP-like processes and changes in sensory experience. The modifications range from growth and shrinkage of pre-established synapses to synaptogenesis, mediated by spine/bouton formation and modest dendritic/axonal rearrangements. The extent of the changes correlate with the level of functional plasticity.

Keywords: structural synaptic plasticity, barrel cortex, experience dependent plasticity

Timing of GABAergic neurons in cortical circuits

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The distributed temporal activity in neuronal circuits of the prefrontal cortex and hippocampus combines emotional information with episodic and spatial memory to guide decisions on behavioural action. In the hippocampus and medial prefrontal cortex of rodents, single neurons of unknown identity exhibit specific firing patterns during spatial decision-making tasks. The cerebral cortex consists of highly diverse neuronal types with distinct synaptic connectivity, molecular expression profile and contribution to network activity. Cortical neurons can be divided into excitatory pyramidal cells, which use glutamate as a neurotransmitter and give both local and long-range axonal projections, and inhibitory interneurons, which are GABAergic and control the activity and timing of pyramidal cells mainly through local axons. These neurons can be further subdivided on the basis of their distinct axo-dendritic arborisations, subcellular post-synaptic targets, and by their differential expression of signalling molecules, including receptors, ion channels, neuropeptides, transcription factors and Ca2+ binding proteins. We have recorded identified GABAergic interneurons in the medial prefrontal cortex of anaesthetised rats during network oscillations using the juxtacellular recording and labelling technique and investigated their contribution to the synchronisation of theta oscillations between the hippocampus and medial prefrontal cortex. Furthermore, we recorded the activity of hippocampal and prefrontal interneurons and pyramidal cells with tetrodes in freely-moving rats during the learning and execution of a matching-to-place task on a continuous T maze. Our results indicate that different GABAergic interneurons release GABA at distinct times to different domains of pyramidal cells explaining the need of diverse classes of interneurons and thereby contributing to the formation of cell assemblies and representations in the hippocampus.

Keywords: interneuron, cell type, network oscillation

Dynamics of the mouse auditory cortex

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In order to learn how sounds are represented in cortical circuits of the mouse auditory cortex, we investigated the coding properties and information content of local neuronal ensembles using in vivo two-photon calcium imaging. We find that population responses to a broad range of auditory stimuli are typically structured in only one or two modes, suggesting attractor-like dynamics at the local scale. Thus, the activity of tenths of neurons can serve as a basis for sound encoding, but the typical scale of fully discriminative representations of sounds is likely much larger. Interestingly, perceptual classification of sounds by mice performing an auditory discrimination task is reflected by the similarity of the respective population responses constructed from multiple local ensemble recordings. To gain a handle on specific elements of cortical circuits, we have generated a transgenic mouse model expressing photoactivatable GFP that allows photolabeling of individual neurons after functional characterization with calcium imaging.

Keywords: calcium imaging, population codes, transgenic mice, photolabeling

Information transformation between cortical regions during a tactile task

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Rats are trained to use their vibrissal sensory system to discrinate among a set of surfaces which vary in inter-groove spacing. They must classify four such textures into two behavioral categories. Neurons in primary somatosensory cortex ('barrel cortex') mainly encode the kinetics of whisker motion along the surface. We are examining the secondary somatosensory cortex and other regions, simultaneously, to determine how the same tactile experience is transformed into a more abstract representation, less correlated with physical properties and more correlated with behavioral significance.

Keywords: tactile, coding, texture, vibrissa, whiskers

Self-inhibition of fast-spiking basket cells: functional roles within cortical microcircuits

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In the neocortex, parvalbumin-positive fast-spiking (FS) basket cells innervate almost exclusively the perisomatic region and proximal dendrites of pyramidal neurons. Therefore, FS-cell GABAergic synapses set the timing of action potentials of many pyramidal neurons, crucially regulating the neuronal output and promoting synchronous discharge of a large population of principal cells. Remarkably, in the neocortex, FS cells make a large number of GABAergic synaptic contacts with themselves (autapses) that modulate their own spike frequency and play a key role in setting their own precise spike-timing. Therefore, precisely timed autaptic self-inhibition might contribute to improve FS-cell synchronous firing during network activity. We have found that, in addition to fast and reliable single spike-dependent autaptic release of GABA, high frequency trains of action potentials in FS interneurons can generate a delayed and prolonged GABAergic self-inhibition due to sustained asynchronous release at FS-cell autapses. Asynchronous release of GABA is simultaneously recorded in connected pyramidal neurons. Functionally, asynchronous release decreases FS-cell spike reliability and reduces the ability of pyramidal neurons to integrate incoming stimuli into precise firing. By switching between two modes of GABA release at their autaptic and synaptic nerve terminals, FS interneurons can effectively filter network activity, behaving as a precise coordinator when activity is normal, but quickly breaking the synchrony pattern when activity levels reaches a certain frequency threshold.

Frequency-dependent inhibition between neocortical pyramidal cells

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The cortical and striatal microcircuits are both characterized by a majority of projection neurons (pyramidal cells and medium spiny neurons, respectively), and a small yet diverse population of GABAergic interneurons. The output of the respective microcircuits depends on the intricate synaptic connectivity, and mainly the interplay between excitatory inputs with feedback and feedforward inhibitory pathways. I will discuss recent findings in neocortex (Silberberg & Markram, Neuron 2007) and striatum (Planert et al, J. Neurosci. 2010) pertaining to these inhibitory pathways and their implication on information processing.

Keywords: GABAergic, interneurons, martinotti cell, fast spiking cell, neocortex, striatum, feedback feedforward inhibition

Interneurons at the end of the functional spectrum

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Neurogliaform cells (NGFCs) are cortical GABAergic interneurons characterized by late spiking firing pattern, α-actinin2, GABAAδ receptor content and unitary volume transmission leading to slow GABAA and GABAB receptor mediated responses. Such functional distinction is expected to be based on the concerted action of several genes active specifically in NGFCs. Quantitative real-time PCR provides opportunity to compare the expression profile of a relatively limited number of markers in cortical interneurons, but data concerning the entire genome using material collected from identified interneurons is limited.

We developed a method in order to analyze the global gene expression profile of NGFCs and applied a harvesting procedure standardized for single cell PCR. Combining whole cell recordings and post hoc light microscopic assessment to identify NGFCs (n = 30) from rats (P22-40), RNA from harvested cytoplasms was amplified and hybridized onto a rat DNA-microarray. We focused our preliminary analysis on genes that are highly expressed in neurogliaform cells but have low or no expression in the controls. Out of 26209 genes we got no, or close to background signal in 5551 and 5094 genes in the case of neurogliaform and cortical cells, respectively. We identified 1143 transcripts that exhibited significantly ($p < 0.001$) higher expression in all NGFCs compared to controls. In addition, we tested the expression profile of 40 out of the 1143 NGFC specific transcripts with quantitative real-time PCR comparing neurogliaform and control samples showing results in line with the microarray technique thus validating the gene chip analysis. Among the NGFC specific transcripts, we confirmed known markers for NGFCs (GABAAδ, α-actinin 2), and, in addition, neurotransmitter receptor subtypes, enzymes and signaling molecules undetected in NGFCs to date. Whole cell electrophysiological experiments and immunocytochemistry confirmed the action of dopamine receptor subtypes not reported previously in NGFCs.

These initial results confirm that global gene expression profiling combined with functional characterization is routinely feasible in identified cortical interneurons. Groups of NGFC specific transcripts are expected to selectively modulate the action of NGFCs in the network and could form the basis of their interaction with neural, glial and vascular elements of the cortex.

Functional roles of neurogliaform cells in superficial layers of the neocortex

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The neocortex is composed of excitatory pyramidal neurons and inhibitory GABAergic interneurons. Depending on their target structure interneurons can be subdivided into soma-, dendrite- and axon-targeting interneurons. Distal apical dendrites of layer 5 pyramidal neurons are capable to generate a regenerative dendritic calcium spike that propagates to the axo-somatic region to trigger action potentials. Direct electrical recordings from distal apical dendrites of layer 5 pyramidal neurons recently demonstrated that deep layer interneurons can block the initiation of dendritic calcium spike in L5 pyramidal neurons. Another type of dendrite-targeting interneuron, neurogliaform cells, has been shown to provide non-specific inhibition to nearby neurons by releasing GABA into the extracellular space. In my talk, I will discuss recent findings on the role of neurogliaform cells in neuronal microcircuits in superficial layers of the neocortex. I will further highlight their roles in controlling dendritic excitability of pyramidal neurons.

Keywords: dendrite, GABA, interneurons, neocortex, neurogliaform cell

Combining EEG and transcranial stimulation in neuroplasticity studies

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The combination of brain stimulation with simultaneous electrophysiological brain imaging, TMS-EEG co-registration, has potential to be of great value for understanding human brain function. It provides real time information about the state of the cortex activity, its functional connectivity, and how brain stimulation modifies such activity and connectivity. Moreover several EEG-TMS studies have explored the possibility of inducing frequency-specific effects by rhythmic TMS to modify behaviour (Klimesch et al., 2005), peripheral responses (Brignani et al., 2008), or to define the relation between the oscillatory state of the cortex and the response to an upcoming stimulus (Romei et al 2008). Given the recent advances in EEG research, the ability to modulate brain activity in the frequency domain is particularly timely and promises to have a huge impact across many domains of clinical and basic neuroscience. In this respect, the possibility to induce neuromodulatory effects by rTMS holds considerable promise not only for advancing our understanding of brain rhythms but also in designing new neurorehabilitation strategies (Miniussi et al., 2008). The present talk will consider what and how new information can be gained by integrating these two approaches to investigate the functional sate, hierarchy and connectivity of cortical brain areas.

TMS-EEG studies of brain excitability and connectivity

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The evolving spatiotemporal pattern of cerebral activity evoked by transcranial magnetic stimulation (TMS) can be recorded with millisecond time resolution using multichannel electroencephalography (EEG). If the location and orientation of the stimulator coil is known with respect to the head, one can calculate the distribution and orientation of the induced electric current in the brain. This allows one to deliver the stimuli to desired neuroanatomic locations in the brain according to individual MRI. Navigated brain stimulation (NBS) can have 3-mm accuracy (rms location error) in targeting the neuron-stimulating electric field. When the TMS-evoked EEG is recorded, one obtains direct measures of cortical excitability and time-resolved area-to-area connectivity. One can also monitor changes in excitability and connectivity in the course of treatment, medication, or rehabilitation. Although the electrical artifact due to the TMS pulse has been largely solved by device manufacturers, one needs special precautions and post-processing to deal with artifacts, in particular those from the TMS-activated muscles. Early examples of TMS-EEG studies include those of Ilmoniemi et al. (1), Komssi et al. (2), and Massimini et al. (3). More recent studies will be presented as well.

References:

1. Ilmoniemi et al., NeuroReport 8, 3537-3540 (1997)
2. Komssi et al., Clin. Neurophysiol. 113, 175-184 (2002)
3. Massimini et al., Science 309, 2228-2232 (2005)

Brain-computer interfaces: basic principles and perspectives

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BCI (the acronym for brain-computer interface), also called BMI (brain-machine interface) is an active interaction between the brain of a subject and an artificial device trough a personal computer. The interaction excludes the brain normal output pathways represented by peripheral nerves and muscles. It could lead to move a cursor on a screen, an artificial limb, or again an electric wheelchair, only with the modulation of specific neuronal features. It results particularly useful for those with chronic neuromuscular diseases, stroke or spinal cord injury.

As a matter of fact, a lot of patients suffering from these situations are in a severely paralyzed state and BCI could give them the only chance to interact with the external environment. In this way they can express their wishes to the medical staff or even to their relatives, or move neuroprostheses.

At the moment, demonstrated prototypes include controlling robots, wheelchairs and prosthetic devices, operating virtual keyboards, internet browsing, navigating in virtual realities and playing games. Main application areas in the near future include motor substitution, entertainment, motor recovery, communication and control. Research will mainly concentrate on co-use of different methods of BCI control, account for individual differences, optimization of user interface, incorporation of artificial intelligence.

Nowadays, it is clear that the development of new BCI strategies is a multidisciplinary problem, involving biology, psychology, engineering, mathematics and informatics. Only the interaction within this research fields will lead to a better understanding of the underlying mechanism of the work in our brain, and so to improve assistance and sustain greater independency for people who cannot move their body anymore.

Brain-computer interfaces: muscle-independent communication for the paralyzed

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The development of different visual and auditory paradigms for Brain-Computer Interfaces (BCIs) based on EEG input signals such as slow cortical potentials, sensorimotor rhythms, and P300 evoked potentials provided the possibility of muscle-independent communication and environmental control. Even so, evaluations of BCIs on clinical user groups are still rare. Patients with severe physical impairment due to neurological diseases such as amyotrophic lateral sclerosis (ALS) or muscular dystrophy lose their ability to communicate by means of speech or assistive communication devices. A meta-analysis (Kübler & Birbaumer, 2008) on 29 patients demonstrated that voluntary brain regulation for BCI control is possible in all stages of paralysis except in the Completely Locked-In State (CLIS). The CLIS refers to a state in which all control over skeletal muscles is lost, and thus, no communication and social interaction is possible any more.

The talk will introduce different BCI applications tested with ALS patients and the development of a new paradigm for CLIS patients which will overcome the problems of voluntary regulation of brain activity and probable attention deficits.

Mitochondrial diseases of the skeletal muscle

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Mitochondrial diseases,described restrictively as disorders due to defect of the mitochondrial respiratory chain, are notoriously heterogeneous, both clinically and genetically. I will review only disorders affecting exclusively or predominantly skeletal muscle (mitochondrial myopathies). As the respiratory chain is under dual genetic control, I will consider first myopathies due to mutations in mitochondrial DNA (mtDNA), distinguishing those due to defects of mitochondrial protein synthesis in toto from those due to mutations in protein-coding genes. I will discuss how defects in the ubiquitous mtDNA can affect specifically skeletal muscle and how a homoplasmic mtDNA mutation can cause a reversible myopathy of infancy with cytochrome c oxidase (COX) deficiency. Moving on to defects in nuclear DNA (nDNA), I will consider separately defects in genes encoding subunits of the respiratory chain ("direct hits"), defect in genes encoding assembly proteins ("indirect hits"), and defects in genes that control the intergenomic "dialogue", especially myopathies due to mtDNA depletion. Separately, I will present evidence that alterations in the mitochondrial membrane lipid milieu cause at least one disease dominated by myopathy, Barth syndrome. Finally, I will touch upon the relatively new area of mitochondrial dynamics (movement, fission, and fusion), although muscle does not seem to be the primary target of defects in this function.

Regulation of the sodium pump in skeletal muscle in conjunction with metabolically altered conditions

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We have a committed interest in understanding the regulation of sodium pump activity and its trafficking in response to altered metabolic state associated with Type 2 diabetes and cardiovascular disease. Skeletal muscle Na,K-ATPase plays a central role in the clearance of potassium from the extracellular fluid, therefore maintaining blood potassium concentration. Na,K-ATPase activity in peripheral tissue is impaired in insulin resistant states. Utilizing an established rat model of insulin resistance (high fat diet), and a swimming exercise protocol we found that impaired skeletal muscle Na,K-ATPase activity in glucose intolerant animals is associated with altered expression and phosphorylation of Na,K-ATPase-interacting protein phospholemman, concomitant with parallel changes in Na,K-ATPase subunits expression and the pump units cell surface abundance. Importantly, we found that alterations in the sodium pump subunits expression precede development of insulin resistance in skeletal muscle. Disturbances in skeletal muscle Na,K-ATPase regulation, may contribute to impaired ion homeostasis in insulin-resistant states such as obesity and Type 2 diabetes.

The nicotinic acetylcholine receptor (nAChR) and the Na,K-ATPase functionally interact in skeletal muscle. We have examined the molecular nature and membrane localization of this interaction. The interaction operates at the neuromuscular junction as well as on extrajunctional sarcolemma. Our results suggest a mechanism by which the nAChR in a desensitized state with high apparent affinity for agonist interacts with the Na,K-ATPase to stimulate active transport. The interaction utilizes a membrane-delimited complex involving protein-protein interactions, either directly or through additional protein partners. This interaction is expected to enhance neuromuscular transmission and muscle excitation.

Keywords: skeletal muscle, Na,K-ATPase, type 2 diabetes, neuromuscular transmission

Effect of neural agrin on the regenerative potential of the human skeletal muscle

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Denervation causes an impairment of the functional activity and atrophy of the skeletal muscle. The cellular and molecular mechanisms of post-denervation atrophy are still in part unknown. The response of muscle to denervation is a multi-factorial phenomenon and includes coexisting processes. Some of them are triggered by the electrical and mechanical inactivation of the skeletal muscle caused by the absence of nerve inputs, others by the absence of neurotrophic factors normally released by the nerve endings.

Satellite cells are the resident cell population responsible for the post-natal maintenance of the skeletal muscle mass. Potentially, they should counteract the post-denervation atrophy. However, the satellite cell differentiation is abortive in denervated skeletal muscle in vivo. The emerging hypothesis is that, the absence of neurotrophic factors could be one of the reasons of the impaired regenerative potential of the satellite cells.

We explored this new aspect of skeletal muscle plasticity. We observed that neural agrin plays a crucial role in the maturation of the excitation-contraction coupling mechanism in developing satellite cells. Our findings provide new insights into the comprehension of the molecular mechanisms of post-denervation atrophy.

Keywords: agrin, calcium homeostasis, myogenesis, satellite cells, skeletal muscle.

Response to hypoxia in the in vitro regenerating human skeletal muscle

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Skeletal muscle injury is followed by regeneration which is characterized by satellite cell activation, myoblast proliferation, formation of myotubes and/or fusion of myoblasts with existing myofibers. Regeneration process is not important only in pathologic context since it also eliminates microscopic injuries incurred during skeletal muscle contractions and is probably at least under some circumstances involved in skeletal muscle hypertrophy in response to exercise.

Skeletal muscle is exposed to hypoxia under various physiological (e.g. exercise and high altitude) and pathophysiological (e.g. due to respiratory disease, anaemia or atherosclerosis) conditions. While transient mild to moderate hypoxia during physical activity might contribute to metabolic and vascular adaptations characteristic of endurance exercise, severe and/or prolonged hypoxia has deleterious effects on muscle function and can result in overt necrosis of skeletal muscle fibers. Moreover, hypoxia might directly affect the regeneration that follows ischaemic necrosis or exercise under hypoxic conditions.

We therefore studied the characteristics of hypoxic, HIF-1α mediated response in human primary myoblasts. Their response to hypoxia is swift, dynamic and largely independent of systemic factors like glucocorticoids. Interestingly, HIF-1α up-regulation in primary human myoblasts is only transient, which could at least partly be due to the negative feedback through the natural antisense transcript aHIF whose level is increased with prolonged exposure to hypoxia.

Keywords: skeletal muscle regeneration, hypoxia, myoblast, HIF-1α, aHIF

Emotion-cognition interactions in schizophrenia: effects of emotional distraction on working memory

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The main aim of this presentation is to introduce novel experimental and meta-analytic results regarding emotional functioning in schizophrenia. Schizophrenia is a heterogeneous neuropsychiatric illness with abnormalities in working memory (WM) and aspects of emotional processing. However, few studies have examined the interaction between emotion and cognition in this illness; specifically the effects of emotional interference on ongoing cognitive operations. Prior behavioral work suggests that schizophrenia may be associated with increased susceptibility to distraction and functional neuroimaging (fMRI) studies have suggested possible neural abnormalities in response to emotional information. However, no study to date has examined effects of emotional and non-emotional interference during minimal cognitiveload and during delayed WM in schizophrenia using fMRI. The present study tested the hypothesis that schizophrenia is associated with a general inability to filter distraction versus a specific deficit in the ability to filter aversive emotional interference.

Concurrent TMS-fMRI investigations to provide direct evidence for top-down prefrontal control in the presence of external interference

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The dorsolateral prefrontal cortex (DLPFC) is recruited during visual working memory when relevant information must be encoded and maintained over the short term and irrelevant, distracting information ignored. The mechanism by which the DLPFC might control the effects of such interference is unclear, and it may either protect relevant memory targets or suppress irrelevant distracters. To adjudicate between these two possibilities, we applied transcranial magnetic stimulation (TMS) during functional magnetic resonance imaging (fMRI), a technique that permits causal assessment of a stimulated brain region's relationship with other regions that are connected under specific behavioural conditions. Participants performed a working memory task requiring the short-term retention of visual stimuli in the presence or absence of visual distracters in which TMS was applied to coincide with the middle of the unfilled delay period or with the distracters. Importantly, when present, distracters were always from the opposite category to memory targets, a manipulation that could reveal the recipient of DLPFC-based control i.e., on regions representing memory targets or regions representing distracters. As predicted, an effect of DLPFC-TMS was evident in posterior areas only in the presence of distracters and this effect was in the form of increased activity in regions representing current memory targets during distraction. No effects of TMS were evident in distracter-representing regions. These results provide novel, causal evidence for a top-down DLPFC-based control mechanism in WM when distraction is present in the visual scene. Moreover, this control appears to target currently relevant over irrelevant information that is being represented in posterior category-specific visual cortex and together, these findings are consistent with a target protection role for prefrontal cortex. I will also present results from a more recent concurrent TMS-fMRI experiment in which the ambiguity of task irrelevant stimuli was varied in order to investigate the nature of DLPFC-based control under more equivocal conditions of distraction. I aim to show that together these studies make a unique contribution to our understanding of the mechanisms by which the DLPFC exerts top-down control including during working memory.

Working memory function in neurodegenerative disorders: a lesson from Huntington's disease

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Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder caused by a trinucleotide repeat expansion in the htt gene on chromosome 4, leading to progressive neuronal degeneration, preferentially but not exclusively within regions of the striatum. Clinically, the disorder is characterized by motor dysfunction, psychiatric symptoms and cognitive deficits. However, accumulating clinical, neuropsychological and neuroimaging evidence indicates that subclinical changes may precede the motor onset of the disease by several years to decades. For instance, individuals who carry the HD mutation, but who remain "presymptomatic" for the motor disturbances (preHD), may exhibit cognitive changes within a range of distinct functional domains, including working memory (WM). Such early phenotypic expressions of HD prior to the onset of manifest motor signs could serve as biomarkers of the disease and hence as surrogate endpoints in future clinical trials. In a series of studies, we have shown that WM dysfunction in manifest HD is associated with altered brain activation of the frontoparietal and the striatum, and that dorsolateral prefrontal function is compromised in preHD individuals during performance of a verbal WM task. In addition, we have shown functional connectivity changes of frontostriatal networks in preHD, and that these changes are modulated by increased WM processing demands. Investigating WM and its neural correlates in manifest HD and preHD may provide a window into the neural architecture of WM function in both healthy conditions and disease. Moreover, probing WM function in HD and preHD may reveal disease-related neural markers which could serve as surrogate endpoints in future interventional trials.

Interference control in visuospatial working memory

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Working memory (WM) refers to a cognitive ability of maintaining and manipulating information over brief periods of time. WM is thought to be comprised of different subcomponents and subserved by dynamic interactions between different brain regions. Two of possible subcomponents - goal maintenance and interference control – are both critical for optimal performance and likely compromised in normal aging, patients with schizophrenia and neurodegenerative diseases. Goal maintenance refers to active maintenance of information in WM, which includes task goals in addition to the specific momentary information represented in WM stores. Conversely, interference control represents mechanisms important for preserving the maintained set from other distracting stimuli, which may be irrelevant for the task. Due to its temporal resolution, electroencephalograhy (EEG) seems to be well suited for observing the temporal dynamics of activity during working memory processes. An ERP component, contralateral delay activity (CDA) is a sustained contralateral negativity present during retention period of working memory task, which both depends on the overall goal maintenance capacities of the individual and closely follows temporary changes in task conditions, like updating of material maintained or presence of distracting stimuli. In present research we used a visual working memory task to study CDA during different kinds of changing working memory conditions and to relate these changes to oscillatory dynamics of the implicated brain network.

Psychostimulant-induced neuroadaptive changes in prefrontal cortex and cognitive dysfunction

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Persisting cognitive and attentional deficits have been reported in psychostimulant addiction, particularly in heavy methamphetamine users. Animal models of cognitive performance after chronic methamphetamine self-administration offers a useful approach to determine methamphetamine-induced cognitive changes and their underlying neurobiology. Here we assessed whether chronic methamphetamine self-administration in rats would produce deficits in attentional set-shifting and object recognition memory. Both of these tasks depend greatly upon intact prefrontal cortex (PFC) function. Therefore, we also determined changes in PFC neuronal activity and several PFC neuronal markers, including levels of monoamine transporters and metabotropic glutamate receptors. Rats consistently showed robust escalation in daily i.v. methamphetamine self-administration over time. Following withdrawal, chronic methamphetamine experienced rats showed selective deficits in the extradimensional set shift component of an attentional set shifting task and deficits in an object-in-place memory task. Recording of PFC neuronal activity revealed a higher basal firing frequency and a significantly greater proportion of burst-firing cells as compared to yoked-saline controls. Furthermore, methamphetamine resulted in reduced PFC mGluR5 receptor expression that correlated with prior methamphetamine intake. These results will be discussed in regards to the relationships between cognitive deficits and the process of psychostimulant addiction, and potential pharmacological interventions to target these deficits.

Modulation of the neurotrophin BDNF in prefrontal cortex by psychotropic drugs

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Brain Derived Neurotrophic Factor (BDNF) is a neurotrophin with a well-established and crucial role in brain plasticity and cellular resiliency. Evidence exists that BDNF is altered in patients with neuropsychiatric disorders, as well as in related animal models of psychopathology. Changes in BDNF, primarily in the prefrontal cortex, may be particularly relevant for depressive and psychotic symptoms, as well as for cognitive deficits. Accordingly, it has been proposed that amelioration of such deficits may occur through psychotropic drug modulation of BDNF function. Here we provide evidence that repeated administration of atomoxetine (ATX) and methylphenidate (MPH), two drugs used to treat attention deficit hyperactivity disorder, regulate BDNF expression through a finely tuned modulation of its transcripts. Repeated administration of ATX increased, while MPH reduced, total and exon IV BDNF mRNA levels in prefrontal cortex. Analysis of BDNF-mediated signaling in the prefrontal cortex revealed that ATX enhanced Akt and GSK3b phosphorylation, whereas MPH reduced the synaptic levels of trkB, the high-affinity BDNF receptor, and ERK1/2 activation. Taken together, these results suggest that modulation of BDNF expression and its transcripts in the prefrontal cortex can be considered a promising target for the modulation of neuroplasticity that may be defective in patients with psychiatric disorders.

Neuroadaptations in amygdala and prefrontal cortex produced by antidepressants

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Antidepressant treatments have been shown to promote different forms of neuronal plasticity, including neurogenesis, synaptogenesis, and neuronal maturation in the hippocampus. We and other laboratories have shown that antidepressants activate signaling of TrkB, the receptor for the neurotrophin brain-derived neurotrophic factor (BDNF) in adult brain, and that BDNF-TrkB signaling appears critical for the behavioral effects of antidepressants in rodents. Neuronal plasticity is active during the early postnatal life, when neuronal networks are modified by environmental guidance, but plasticity is much more restricted in adult brain. We have shown that chronic antidepressant treatment reactivates critical period-like plasticity in the visual cortex of adult rats and, when combined with environmental manipulation, antidepressant treatment can bring about changes in the structure of visual neuronal networks. These effects were associated with increased BDNF and could be inhibited by blocking BDNF signaling through TrkB receptors, emphasizing the important role of BDNF in adult plasticity. We have recently observed that the antidepressant, fluoxetine, reactivates a developmental-like state in the fear-conditioning circuitry, including the amygdala and prefrontal cortex. Fluoxetine treatment increases BDNF expression and induces structural changes reminiscent of early postnatal period in the network comprising amygdala and prefrontal cortex. When combined with fear extinction training, these responses lead to long-term erasure of the conditioned fear response. Our data suggest that pharmacological agents, such as antidepressants, can reactivate developmental-type cortical plasticity and help to repair neuronal networks abnormally wired by adverse early experiences, when antidepressant treatment is combined with environmental rehabilitation in many parts of the cerebral cortex.

The role of cortical mGluRs in methamphetamine-induced memory deficits

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Methamphetamine (Meth) is a highly addictive psychostimulant that causes a spectrum of neurocognitive and motivational impairments in chronic abusers of this drug. While some of these deficits may recover over time, deficits in memory function are the most pronounced and persistent. As such, Meth-induced memory deficits may interfere with successful recovery and increase the risk of relapse. Therefore understanding underlying neurobiology of Meth-induced memory deficits is critical for developing novel treatment strategies and increasing success rate of current treatment approaches. We used extended Meth self-administration, a translational animal model of Meth addiction, to study enduring neuroadaptations in metabotropic glutamate receptors (mGluRs) in the rat cortical and limbic regions in relation to deficits in object recognition memory. Our findings demonstrate that extended Meth self-administration results in impaired performance in 'novel object' and 'object-in-place' recognition memory tasks which persists for up to 4 weeks post Meth exposure. Novel object recognition requires intact connectivity between perirhinal cortex and the hippocampus, while performance in object-in-place memory task brings 'on-line' also the prefrontal cortex. We have studied the changes mGluR subtypes abundant in these regions one day following the memory test. We found that extended Meth self-administration decreased the number of mGlu5 receptors present in synaptosomal membranes isolated from the perirhinal and prefrontal cortices within 1-2 weeks post Meth exposure. Extended access to Meth also decreased surface levels of mGlu2/3 (but not mGlu7) receptors in the prefrontal cortex. Systemic pretreatment with a novel positive allosteric modulator of mGluR5s – CDPPB (30mg/kg) reversed Meth-induced deficits in novel-object recognition memory. Furthermore, increasing the tone on mGlu2/3 receptors using a positive allosteric modulator – LY487379 (25mg/kg) reversed Meth-induced memory deficits in object-in-place task. It is also significant that administration of Modafinil (Provigil) an analeptic drug with partial effects on mGluR2/3 receptors had the same pro-cognitive effects as LY-compound. In summary, our data suggest that reversing glutamatergic deficits by modulation of mGlu2/3 or mGlu5 receptors may represent a promising treatment of cognitive dysfunction in Meth addiction.

Keywords: cognitive, cortex, glutamate, methamphetamine, prefrontal

Ensemble activity of topographically aligned relay and reticular cells in the thalamus

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During natural sleep and anesthesia, neocortical activity is characterized by periods of synchronized activity alternating with periods of silence (up- and down-states). Thalamus is the most important input source and output target of the cortex, yet the relationship between cortical and thalamic activity is not yet fully explored.

To investigate thalamocortical synchrony, we performed simultaneous multiple single-unit recordings in rats under urethane anesthesia in the somatosensory VPM nucleus and the S1 cortex. The recordings were made using four-shank silicon probes, with 200 micron shank separation.

In the VPM two types of spikes were recorded. Wide spikes showed the characteristic burst pattern of relay cells, narrow spikes fired longer and slower bursts. During thalamic spindle episodes the two types of spikes fired at different phases, wide spikes preceded narrow spikes by ~20 ms. Most of the VPM multiunit activity was organized into spindles episodes, which only occasionally coincided with cortical spindles. These thalamic spindles were usually restricted to one or two electrode shanks.

Considering that VPM contains only relay cells and that all features of the narrow spikes are identical to neurons of the thalamic reticular nucleus (nRt), we propose that narrow spikes represent action potentials generated by axon terminals originating in the nRt.

Our data show that in this preparation network activity in VPM is organized into spatially restricted spindle episodes. This indicates that cortical control over VPM is not so strong and reciprocal interaction between relay cells and nRt largely determines network activity.

Using neural ensemble recordings to elucidate circuit dysfunction in the Parkinsonian basal ganglia

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The precise temporal structure offered by synchronized oscillations might be important for appropriate encoding of information in neuronal networks. Thus, disturbances in network oscillations could also underlie dysfunction in disease. For example, inappropriately synchronized beta oscillations (15-30 Hz) in the subthalamic nucleus (STN) accompany movement difficulties in idiopathic Parkinson's disease (PD). The cellular and network substrates underlying these exaggerated beta oscillations are unknown but activity in the external globus pallidus (GP), which forms a reciprocally-connected network with STN, might be of particular importance. I will highlight how we are using neural ensemble recordings in a clinically-relevant rat model of PD to give valuable new insights into the mechanisms underlying these inappropriate oscillations. I will demonstrate that oscillatory activity in STN-GP neuronal networks becomes excessively and selectively synchronized at beta frequencies in a spatially widespread and brain state-dependent manner after lesion of dopamine neurons. Exaggerated beta oscillations are expressed at the levels of single neurons, small ensembles, and much larger populations in STN and GP, and are associated with overall increases and decreases in the firing rates of STN and GP neurons, respectively. The precisely-timed discharges of STN and GP neurons indicate that rhythmic sequences of recurrent excitation and inhibition in the STN-GP network, and lateral inhibition between GP neurons, could actively support abnormal beta oscillations. Thus, ensembles of GP neurons, by virtue of their spatiotemporal synchronization and feed-back/feed-forward mechanisms, are well placed to orchestrate and propagate exaggerated beta oscillations in the STN and, indeed, the entire basal ganglia in PD.

Encoding and reactivation of spatial memory traces by hippocampal cell assemblies

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We recorded place cell activity in behaving rats during a matching-to-multiple-places task that requires frequent updating of memories for goal locations. The task took place on a cheeseboard maze where rats had to find a set of hidden rewards. The place-related firing patterns in CA1 region reorganised to over-represent the learnt goal locations whereas such reorganisation did not take place in CA3. Moreover the learning-related CA1 population firing patterns representing learnt locations predicted memory performances in subsequent memory retention tests. At the goal locations 200Hz high-frequency network oscillations, called sharp-wave/ripples (SWR) were observed. Place cells encoding goal locations increased their firing rate during these SWRs. Moreover, SWRs facilitated the synchronisation of place cells encoding the same goal locations, hence promoting the stabilisation of new place representations. During sleep period following learning, the firing patterns of goal-encoding cells exhibited stronger reactivation than other place cells and their reactivation predicted subsequent memory performances. Altogether, these results suggest that the reorganisation and reactivation of goal-related population firing patterns sustain spatial learning and memory retention abilities.

Structure of neuronal population activity in auditory cortex

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Recordings of single neurons have yielded great insights into how sensory information is represented in the neocortex. However, any one neuron functions as part of a population whose combined activity underlies cortical information processing. Here we review some results obtained by recording simultaneously from auditory cortical populations and individual morphologically identified neurons, in urethane-anesthetized and unanesthetized passively listening rats. Auditory cortical populations produced structured activity patterns both in response to acoustic stimuli, and spontaneously without sensory input. Population spike time patterns were broadly conserved across multiple sensory stimuli and spontaneous events, exhibiting a generally conserved sequential organization lasting for approximately 100ms. Both spontaneous and evoked events exhibited sparse, spatially localized activity in layer 2/3 pyramidal cells, and densely distributed activity in larger layer 5 pyramidal cells and putative interneurons. Laminar propagation differed however, with spontaneous activity spreading upward from deep layers and slowly across columns, but sensory responses initiating in presumptive thalamorecipient layers, spreading rapidly across columns. In both unanesthetized and urethanized rats, global activity fluctuated between "desynchronized" state characterized by low amplitude, high-frequency local field potentials and a "synchronized" state of larger, lower-frequency waves. Computational studies suggested that responses could be modelled by a simple dynamical system model fitted to the spontaneous activity immediately preceding stimulus presentation, reflecting a nonlinear self-exciting system in synchronized states and an approximately linear system in desynchronized states.

Identification of neuronal and molecular targets for antiparkinsonian and antipsychotic drugs

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The basal ganglia are affected by several neuropsychiatric and neurodegenerative diseases, including Parkinson's disease (PD) and schizophrenia. The use of antiparkinsonian and antipsychotic drugs is limited by the emergence of motor side-effects, particularly after prolonged use. The medium spiny neurons (MSNs) of the striatum are an ideal tool to investigate the molecular bases of these disorders. MSNs can be distinguished based on their connectivity to the output nuclei of the basal ganglia and on their ability to express dopamine D1 receptors (D1Rs) or dopamine D2 receptors (D2Rs). Activation of D1Rs promotes glutamatergic transmission, thereby increasing the excitability of striatonigral MSNs. In contrast, activation of D2Rs reduces glutamatergic transmission and inhibits striatopallidal MSNs. This presentation describes the effects produced by L-DOPA, a common antiparkinsonian medication, and haloperidol, a typical antipsychotic drug, on D1R- and D2R-expressing MSNs. Changes in cAMP, extracellular-signal-regulated kinases and mammalian target of rapamycin signaling will be discussed with regard to their cellular localization, functional role and pathological relevance.

The Ras-ERK signalling pathway in the control of hyperdopaminergic disorder

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Hyperdopaminergic disorders include addictive behaviour in response to psychostimulants and L-dopa induced dyskinesia in Parkinson's Disease patients. They are characterised by abnormal cellular changes in the basal ganglia system and in particular in the striatum. Among the intracellular signalling cascades that are found altered in these two brain diseases, the Ras-ERK pathway seems to play a key role in the pathogenesis. Here it will be demonstrated that Ras-GRF1, as a striatal integrator of dopamine and glutamate signals to Ras and ERK, is not only essential for generating the abnormal behavioural responses to cocaine but also for producing motor symptoms associated to chronic L-dopa treatment in a mouse model of Parkinson's Disease. Experimental evidence supporting a therapeutic approach for these brain diseases based on Ras-ERK inhibition will also be provided.

Pharmacological and genetic modulation of signalling pathways improves L-dopa induced dyskinesia: RGS, GRK and PSD-95

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Parkinson's disease is primarily caused by degeneration of dopaminergic neurons in the substantia nigra and the consequent deficit of dopamine in the striatum. Dopamine replacement therapy with the dopamine precursor L-DOPA is the mainstay of current treatment. However, after several years the patients develop L-DOPA-induced dyskinesia, or abnormal involuntary movements, likely due to excessive signaling via dopamine receptors in general and dopamine D1 receptor in particular. In this context, multiple targets and pathways may be amenable to development of gene therapy approaches for Parkinson's disease. The presentation will highlight comprehensive programs from the speaker's laboratory that feature target identification, target validation and proof-of-concept preclinical studies in disease-relevant animal model. Three targets will be highlighted, namely RGS9-2, GRK6 and PSD-95. Such combination constitutes the required preclinical package before embarking into clinical development. While gene therapy has been mostly used so far for enhancing the expression of the target gene, the use of dominant negative or shRNA opens new possibilities. This, combined with the key feature of gene delivery that offers access to intracellular signaling pathways, is likely to further expand the number of proposed targets to be studied.

Experimental models of dopamine-related dysfunctions

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The catecholaminergic neurotransmitter dopamine is involved in a variety of physiological functions ranging from voluntary movement and reward to hormonal regulation and hypertension. Pharmacological agents targeting dopaminergic neurotransmission have been clinically used in the management of several neuro-psychiatric disorders, such as Parkinson's disease, schizophrenia, bipolar disorder and attention deficit hyperactivity disorder (ADHD). To understand the role of aberrant dopaminergic transmission in the pathology of brain disorders several genetic mouse models of hyperdopaminergia and hypodopaminergia have been developed. Recent progress in understanding the complex biology of dopamine receptor-related signal transduction mechanisms has revealed that, in addition to their primary action on cAMP-mediated signaling, dopamine receptors can act through diverse signaling mechanisms that involve alternative G protein coupling or through G protein-independent mechanisms via interactions with ion channels or proteins that are characteristically implicated in receptor desensitization, such as β-arrestins. In particular, genetic models of dopamine-related dysfunction were instrumental to identify the role of Akt/GSK-3 signaling cascade in the D2 dopamine receptor mediated behaviors and functions. Investigations performed in mice with genetically enhanced or reduced dopaminergic transmission will be discussed.

Classification and basic pathology of Alzheimer disease

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The lesions of Alzheimer disease include accumulation of proteins, losses of neurons and synapses, and alterations related to reactive processes. Extracellular Aβ accumulation occurs in the parenchyma as diffuse, focal or stellate deposits. It may involve the vessel walls of arteries, veins and capillaries. The cases in which the capillary vessel walls are affected have a higher probability of having one or two apoε 4 alleles. Parenchymal as well as vascular Aβ deposition follows a stepwise progression. Tau accumulation, probably the best histopathological correlate of the clinical symptoms, takes three aspects: in the cell body of the neuron as neurofibrillary tangle, in the dendrites as neuropil threads, and in the axons forming the senile plaque neuritic corona. The progression of tau pathology is stepwise and stereotyped from the entorhinal cortex, through the hippocampus, to the isocortex. The neuronal loss is heterogeneous and area-specific. Its mechanism is still discussed. The timing of the synaptic loss, probably linked to Aβ peptide itself, maybe as oligomers, is also controversial. Various clinico-pathological types of AD have been described, according to the type of the lesions (plaque only and tangle predominant), the type of onset (focal onset), the cause (genetic or sporadic) and the associated lesions (Lewy bodies, vascular lesions, hippocampal sclerosis, TDP-43 inclusions and argyrophilic grain disease).

The fatal dialog between chronic neuroinflammation and tau neurodegeneration

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Neurofibrillary degeneration and neuroinflammation play a fundamental role in the progression of Alzheimer's disease. Previously, it has been clearly demonstrated that microglial activation was correlated with tau burden in Alzheimer's disease and other human tauopathies. We have previously showed that expression of non-mutated human truncated tau derived from sporadic Alzheimer's disease induced neurofibrillary degeneration in the brain of transgenic rats. The neurodegenerative lesions promoted inflammatory response manifested by upregulation of several immune-molecules and morphological activation of microglial cells. Interestingly, the immune response driven by neurodegeneration was significantly determined by genetic modifiers. Two independent transgenic lines expressing the same human truncated tau protein in either spontaneously hypertensive rat (SHR) or Wistar-Kyoto (WKY) genetic background revealed that the immune response driven by tau neurodegeneration was significantly determined by genetic modifiers. In order to determine the molecular mechanism underlying innate immune response induced by misfolded truncated tau, we activated microglia cells with purified recombinant truncated tau. We found that misdisordered truncated tau induced morphological transformation of microglia from resting into the reactive phenotype and simultaneously it induced release of nitric oxide (NO) and pro-inflammatory cytokines (IL-1, IL-6, TNF). Molecular analysis showed the significant involvement of the p38 and p42/44 (ER1/2) mitogen-activated protein kinases pathways in tau-induced microglial activation. Our results showed that misfolded tau induces neuroinflammation which in turn can modify the progression of the disease. These findings suggest that brain immune response is a potent disease modifier of neurofibrillary degeneration.

Are tau proteins only microtubule-associated proteins? Toward a role in nucleus and plasma membrane

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Tau transgenic mice are valuable models to investigate the role of tau protein in Alzheimer's disease and other tauopathies. Compared to other tau Tg mouse models, the THY-Tau22 model shows early onset tau pathology starting at 3 to 6 months of age. Furthermore, it is one of the first models displaying the main features of AD-like tau pathology in the absence of any motor deficits. We report here that in THY-Tau22 mice tau was hyperphosphorylated at several sites as seen in AD brain, and that these mice displayed hippocampus-related impairment in learning and memory and synaptical plasticity. This model is used as an experimental tool in studies to investigate mechanisms underlying cognitive deficits during pathogenic tau aggregation and develop therapeutic strategies.

Neuroendocrine – immune interactions in the pathogenesis of neurodegeneration

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The etiopathogenesis of Alzheimer's disease (AD) as a most serious tauopathy still remains unknown. Although it is believed that stress represents a serious risk factor of AD, exact molecular link between the stress and neurodegeneration is still missing. We have investigated the influence of acute and chronic stressors in intact animals (WT) and mice deficient in CRH. Specifically, we have analyzed the levels of hyperphosphorylated, AD-specific epitopes on the molecule of tau protein, which is the major molecular player in AD type of neurodegeneration. Immobilization stress (IMO) induced a transient hyperphosphorylation of tau proteins in WT and also in CRH (-/-) mice. Tau protein phosphorylation was quantified in several AD specific epitopes (pS202/pT205, pT181, pS396-pS404). The changes were similar in several brain regions: frontal and temporal cortices, hippocampal C1 region, dentate gyrus, and amygdala. Different response has been observed in brainstem nuclei such as Locus coeruleus, Substantia nigra and Raphe nucleus. The strongest tau phosphorylation was observed during 30 minutes long IMO stress. The IMO in duration of 120 minutes followed by 3 hours of rest lead to the complete disappearance of tau protein hyperphosphorylation. Interestingly, the magnitude of pathological phosphorylation of tau protein in CRH (-/-) mice was much lower in comparison to WT animals, which indicates the role of CRH and glucocorticoids in pathogenesis of dementia. Our results strongly suggest that pathological phosphorylation of tau protein induced by stress may lead to misfolding of tau protein and eventually to initiation of neurodegeneration. The CRH plays an important role in stress induced phosphorylation of tau protein, which might be a direct effect of CRH innervations or an effect mediated via HPA axis.

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Alzheimer's disease: present and future treatment strategies

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Alzheimer disease (AD) is the most common cause of dementia in advanced age. Currently available medications improve AD symptoms, and development of disease-modifying drugs is a very active area of research, which includes cholinergic, anti-amyloid compounds, drugs targeting tau-protein or mitochondria, neurotrophins and other therapeutic approaches.

The amyloid cascade hypothesis dominates current drug development strategies, but whether Aβ is more pathognomonic than pathogenetic is not yet clear, and so is the therapeutic role of Aβ removal.

Identification of effective disease-modifying drugs will benefit from understanding the interplay between mechanisms causing neurodegeneration in AD. Combined therapy could be a more effective strategy to halt AD progression. Solving methodological problems in clinical trials on AD - including use of standardised diagnostic criteria able to identify homogeneous group of patients, appropriate treatment duration and measures of disease-modifying effects - will help finding a cure for AD.

The lecture will summarise current treatment possibilities for AD, as well as the main findings for new, and less new drugs with novel therapeutic use in AD, focusing mainly on compounds in the human testing phase.

Candidate neurophysiological markers of Alzheimer's disease

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The research field of clinical neurophysiology of neurodegenerative diseases is expanding. Electroencephalography (EEG) is a method of interest due to its non-invasiveness and simple logistics. Time resolution in milliseconds reflects processes at the synapse level and makes the EEG a method of choice to study disturbances in brain function. Computerized analysis of EEG signal has an advantage of being more objective and quantitative way of data analysis which overcomes limitations of visual interpretation and produces variables informative of various aspects of brain function. EEG signal could be disentangled in frequency, time and space domain by analysis of power spectra, microstates, dipole source strength and localization, coherence and global field synchronization. Increase of EEG power in slow and decrease in fast frequency bands is consistent finding in patients with Alzheimer's disease (AD) and shows good correlation with measures of general cognition. Shorter microstates over larger areas were found in AD patients, a finding which probably reflects lack of stable brain states necessary for normal cognitive function. Method of EEG source localization is unambiguous localization of sources of EEG activity in different frequency bands and shift of normal alpha activity from parieto-occipital cortex towards frontal regions was found in preclinical-AD patients with only mild cognitive impairment (MCI). Decreased EEG coherence and Global Field Synchronisation (GFS) reflect neurofunctional disconnection and show correlation with a gradient of increasing cognitive impairment in AD. These candidate neurophysiological markers should be validated in clinical settings with regard to their supplementary diagnostic and prognostic value in individual patients.

CSF biomarkers in Alzheimer's disease, Parkinson's disease and atypical parkinsonism

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Biomarkers that are related to the pathophysiology of Alzheimer's disease (AD) may help to detect the preclinical stages of disease, and improve early and differential diagnosis. Here, we provide an overview of current literature on the core AD biomarkers, Aβ and phosphor-tau (p-tau), on different methods and modalities of assessing them [e.g., cerebrospinal fluid (CSF) analysis and on their diagnostic and predictive value in preclinical and clinical stages of AD. While fairly accurate, the clinical diagnosis of probable AD based on standard diagnostic criteria does not take into account the long preclinical and prodromal course of AD. AD-related pathophysiological changes can occur many years and even decades before the appearance of clinical dementia syndrome. CSF levels of Aβ42, tau, and hyperphosphorylated tau protein (p-tau) can distinguish subjects with mild cognitive impairment (Aβ and phosphor-tau (p-tau)) who are likely to progress to AD. They also show preclini cal alterations that predict later development of early AD symptoms. Also, there are reports showing that combinations of these biomarkers could differentiate Parkinson's disease patients not only from normal controls but also from patients with AD and atypical parkinsonismus cases. In this report, the CSF biomarkers analysis from the patients attending the Memory clinic in Belgrade, Serbia will be presented. Our experience showed that the analysis of CSF- biomarkers in patients with MCI, AD, PD and atypical parkinsonismus is reliable, sensitive and reproducible.

Keywords: Alzheimer's disease, Aβ, phosphor-tau (p-tau), total tau, MCI, Parkinson's disease, atypical parkinsonism

Neurodegenerative syndromes which manifest with parkinsonism and dementia

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Different neurodegenerative diseases present with a variety of clinical symptom combinations. It is not clear if these syndromes are different nosological entities or they are just a different expression of the same disease. Dementia with Lewy bodies (DLB) has recently been identified as a separate disease but diagnosis can be difficult, in particular the differentiation from related dementias of Alzheimer's disease (AD) and Parkinson's disease with dementia (PDD). Careful cognitive assessment may aid differential diagnosis between these different types of dementia and provide theoretical insight into the nature of the underlying impairments. The lecture will report on a clinical study focused on neurodegenerative syndromes manifesting with parkinsonism and dementia based on the results of neurological, psychological and complex imaging investigations. Previous studies have shown that DLB is associated with profound impairment in both spatial and perceptual networks of visual information processing, but studies comparing all three syndromes for visuospatial impairment are lacking and the available results are sometimes controversial. The visual-perceptual and visual-constructional performance compared to imaging results in patients with early stage DLB, AD and PDD will be stressed.

The lecture will summarize available and clinically relevant results from the study and discuss the utility of the results in the process of the differential diagnosis of neurodegenerative diseases which present as a combination of dementia and parkinsonism.

Structural and functional diversity of the intercalated cell masses of the amygdala and implications for fear learning

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Although extinction-based therapies are amongst the most effective treatments for anxiety disorders, the neural bases of fear extinction remains still largely unclear. Recent evidence suggests that the intercalated cell masses of the amygdala (ITCs) are critical structures for fear extinction. However, the neuronal organization of ITCs and how distinct clusters contribute to different fear states are still entirely unknown. I will elucidate the cellular organization and efferent connections of one of the main ITC clusters in mice. Our data show an unexpected heterogeneity in the axonal pattern of medial paracapsular ITC (Imp) neurons and the presence of three distinct neuronal subtypes. Functionally, we observed that the Imp is preferentially activated during fear expression whereas extinction training and extinction retrieval activate the main ITC nucleus (IN), as measured by quantifying immediate early gene expression. This can be explained by the GABAergic monosynaptic innervation of IN neurons by one subtype of Imp cells, namely the medial capsular projecting (MCp)-Imp neurons. MCp-Imp neurons also target large ITC cells that surround ITC clusters. Our results reveal a distinctive participation of ITC clusters to different fear states and the underlying anatomical circuitries, hence shedding new light on ITC networks and providing a novel framework to elucidate their role in fear expression and extinction.

Keywords: amygdala, fear, intercalated cell masses, GABA, neuronal networks

Perisomatic inhibition in the basolateral amygdala and its control by inputs from basal forebrain

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Neuronal networks in the basolateral amygdala (BLA) are suggested to be involved in assigning affective value to sensory stimuli. The low spiking probability of amygdalar projection neurons (PCs) recorded in vivo should be significantly increased to enable them to respond appropriately to sensory signals. Thus, perisomatic inhibition that effectively controls the firing activity of PCs is likely a subject of profound modulation. In the microcircuitries of BLA perisomatic inhibition originates from three types of local GABAergic cells: fast spiking basket cells, axoaxonic cells and regular spiking basket cells. The basal forebrain that heavily innervates the BLA via cholinergic and GABAergic afferents has significant impact on the operation of the perisomatic inhibition. On one hand, the output of the three GABAergic cells could be substantially reduced by cholinergic receptor activation. On the other hand, GABAergic afferents from basal forebrain selectively target these GABAergic cells in the BLA as well. Consequently, perisomatic inhibition in the BLA is organized similarly to that found in other cortical areas and its function is substantially controlled by the basal forebrain.

Keywords: GABA, synaptic inhibition, neuronal networks, interneurons, acetylcholine, cannabinoid

Neuropetide S: control of state-dependent properties in the amygdala in instances of stress and fear

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Fear is a crucial adaptive component of the behavioral repertoire that is generated in anticipation of or in response to stimuli which threaten to perturb homeostasis. Fear-relevant associations can be learned and consolidated as part of long term memory, and be extinguished through extinction learning. Acute or chronic stress can severely influence the balance of these processes, eventally leading to pathological alterations and anxiety disorders. Recent years have seen considerable progress in identifying relevant brain areas - such as the amygdala, the hippocampus and the prefrontal cortex - and key neuromodulatory mechanisms involved in balancing physiological and pathophysiological states of fear and anxiety. The recently discovered Neuropeptide S (NPS) and its cognate receptor represent such a system of neuromodulation. On one hand, NPS increases wakefulness and arousal. On the other, NPS produces anxiolytic-like effects by reducing acute fear responses and by modulating long-term aspects of fear memory, like contextual fear and fear extinction. Underlying mechanisms involve the presynaptic control of excitatory transmitter release, particularly in synaptic contacts to defined subsets of GABAergic interneurons in the amygdala. The recent availability of a NPS-EGFP transgenic mouse line has revealed that NPS-expressing neurons in the brainstem possess distinctive intrinsic properties, respond in an excitatory fashion to corticotrophin releasing factor (CRF), and send axonal projections to widespread but defined targets in the subiculum, intralaminar thalamus and amygdala. Data from combined behavioural and electrophysiological in vivo and ex vivo studies indicate that the NPS system is activated in stressful situations, controls hyperexcitability in the amygdala and can thereby overcome stress-induced impairment of fear extinction. Overall, the NPS system appears to be critically involved in state-setting properties of network functions in the amygdala in instances of stress and fear.

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Keywords: neuropeptide S, fear, amygdala, neurotransmitter release

Neuropeptide Y modulates fear, anxiety and depression-like behavior in distinct nuclei of the amygdala

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Fear and anxiety are integrated in the amygdaloid nuclei and involve the interplay of the amygdala with various other brain areas. Neuropeptides play a critical role in regulating these processes. Neuropeptide Y (NPY), a 36 amino acid peptide, is highly expressed in limbic brain areas, including the amygdala. Depending on the receptor subtypes involved (Y1, Y2 or Y4), NPY has different, in part opposing effects on anxiety, fear and depression-related behaviors. We combined site-specific deletion of NPY receptors and locally restricted over-expression of NPY receptor subtype selective ligands with behavioral analysis to elucidate the contribution of the individual receptor subtypes in the modulation of emotional behavior. We are proposing that NPY mediates its action not only by stimulation of post-synaptic receptors but also by the presynaptic inhibition of GABA and/or NPY release. I will present data supporting an inhibitory role of NPY in the basolateral amygdala on anxiety as well as in the processing of fear, while NPY in the central amygdala is predominantly modulating anxiety and depression-like behaviors.

Keywords: neuropeptide Y, amygdala, fear, anxiety

Development of associative pathways in the human brain

Ivica Kostović

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The earliest connections between cortical neurons begin during the early fetal period in the form synaptic-junctional oscillatory network distributed within the specific laminas above and below cortical plate. Long association pathways develop very early (by mid-fetal period) in the limbic cortex (hippocampus, gyrus cinguli, orbital cortex, peri-amigdaloid cortex). On the contrary, neocortical long pathways develop later, during late fetal period in the deep portion of the subplate zone along external capsula (external sagittal stratum). Short association pathways between adjacent cortical areas and gyri develop earlier in the limbic cortex than in neocortex. Neocortical short cortico-cortical pathways develop during first postnatal year of life. Nothing is known about development of short cortico-cortical and intracortical pathways during early childhood, childhood and adolescence. However, imaging studies and histological analysis of myelinization indicate that postnatal period is important for development of short cortico-cortical connectivity at the level of gyral white matter and intracortical fibrilar organization (radii). The interface between gyral white matter and layer VI is the site of major reorganization during postnatal period and involves also U-fibers. In conclusion, earliest connectivity forms framework for endogenous oscillations. Cortico-cortical pathways of the limbic cortex, as well as long association pathways of neocortex, develop their trajectories in utero, controlled by genetic mechanisms. There is developmental disconnection between limbic (emotional) and neocortical (cognitive) cortico-cortical connectivity. Postnatal development of short cortico-cortical connectivity opens possibility of developmental interactions with environmental influences.

Keywords: cortico-cortical, connectivity, association pathways, neocortex, limbic cortex, development

Molecular evolution and development of neural circuits of the cerebral cortex

Kyle Meyer

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The development of the human brain is one of the crowning achievements of evolution as well as one of its biggest unsolved mysteries. What distinguishes humans from other species is largely thought to reside in the unique features of brain development, especially in how highly complex neural circuits of the cerebral cortex are wired. However, in addition to giving us remarkable cognitive and motor abilities, the formation of intricate cortical neural circuits may have also increased our susceptibility to neuropsychiatric and neurodegenerative disorders. How genes shape the development and evolution of neural circuits in the human cerebral cortex will be discussed.

Extraordinary neoteny of the human prefrontal cortex: massive synaptic pruning on main projection neurons extends to third decade

Zdravko Petanjek

Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Croatia

The major mechanism for generating diversity of neuronal connections beyond their genetic determination is the selective stabilization of the supernumerary synapses that had been proposed more than four decades ago. Selective stabilization assumes that during a period of overproduction of synapses, neuronal activity tunes the molecular structure of individual synapses and determines which ones will be removed from the network. The selective stabilization hypothesis gains support by the discovery that synaptic connections in the cerebral cortex of human and non-human primates are initially overproduced about twice in number and are pruned during puberty to reach the adult level at the onset of adolescence. However, recent brain imaging studies show the functional, structural and molecular maturation of connections, including their regression, might continue through adolescence and young adulthood, but direct cellular evidence is lacking. We provide the first evidence that developmental remodeling of dendritic synaptic spines on identified pyramidal neurons in the human prefrontal neocortex, including their transient overproduction and selective elimination, passes beyond the period of adolescence and continues throughout the third decade of life, before stabilizing to the adult level. This strongly supports the view that protracted cognitive and emotional development observed at that age depends predominantly on structural network reorganization, including elimination of supernumerary synapses and, not only on fine molecular tuning of stable neuronal connections. Those findings have implications for delineating the critical period and understanding the mechanisms of environmental impacts such as education and training on development of human cognitive capacities, and also, provide insight into the pathogenesis of late onset, human-specific, neuropsychiatric disorders.

Keywords: associative cortex, cortico-cortical connections, principal neurons, working memory, human specific psychology, schizophrenia, emerging adulthood

Sunday, 25 September, 08:30
[Symposium: Lifetime development and reorganization of cortical circuitry]

Association of cortical thickness and cognitive ability in children and adolescents

Sherif Karama

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In the last few years, a number of reports have shown that scores on various tests of cognitive ability are correlated with regional brain structure and function. In this presentation, the widely used psychometric framework for understanding cognitive ability difference will first be provided and then followed by a summary of pertinent findings pertaining to the association between general cognitive ability and cortical structure. More specifically, associations between general cognitive ability and cortical thickness, cortical surface area, cortical volume, and degree of gyrification will be summarized and discussed. The presentation will have a developmental perspective as most findings that will be presented will stem from an examination of the NIH Study of Normal Brain Development; a longitudinal study of children and adolescents (age 4.3 to 18 years, n = 433) representative of the general US population for that age range (US 2000 census).

Molecular architecture of synaptic endocannabinoid signaling in the brain

István Katona

Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

Endocannabinoids are well known lipid mediators controlling neurotransmitter release throughout the brain, which leads to several forms of short-term or long-term synaptic plasticity. A major paradox however is that molecular species of endocannabinoids are chemically heterogeneous raising the possibility that distinct endocannabinoid pathways may have been evolved to fulfill different physiological functions. In the present introductory talk, recent and new findings will be summarized to provide a detailed picture on the subsynaptic segregation of several molecular players of the endocannabinoid system. I will illustrate that distinct endocannabinoid signaling pathways are compartmentalized at the subcellular level suggesting a functional division of labor between given endocannabinoid, or endocannabinoid-like molecules.

Keywords: CB1 cannabinoid receptor, 2-arachidonoylglycerol, synapse, diacylglycerol lipase, anandamide

Sunday, 25 September, 10:15
[Symposium: The "grass roots" of plasticity in the brain: endocannabinoids as key regulators of synapses, networks and behaviors]

Astrocytes control spike-timing dependent plasticity at cortical synapses

Thomas Nevian

Department of Physiology, University of Bern, Switzerland

Spike-timing dependent plasticity (STDP) is a plausible cellular mechanism for activity-dependent modification of synaptic strength. The magnitude and direction of synaptic strength depends on the precise timing of pre- and postsynaptic spikes. Presynaptic spikes followed by postsynaptic spikes within a time window of up to 25 ms results in long-term potentiation (LTP), whereas presynaptic spikes preceding postsynaptic spikes within a slightly broader time window of 50 ms result in long-term depression (LTD). STDP has been described at many different synapses revealing that the underlying molecular mechanisms at a particular synapse can be quite diverse. We have investigated the molecular mechanisms of STDP at layer 4-to-layer 2/3 synapses onto pyramidal neurons in the somatosensory cortex of rats. It is known that LTD at this synapse requires retrograde signaling by postsynaptically synthesized endocannabinoids to the presynaptic site. Nevertheless the exact retrograde signaling mechanism is still unknown. We found that by interfering with astrocytic calcium signaling LTD could be blocked. Therefore we suggest that astrocytes in close proximity to the activated synapse are a critical element in mediating the induction of LTD.

Keywords: spike-timing dependent plasticity, STDP, endocannabinoid, barrel-cortex, synaptic plasticity, astrocyte, calcium

Dendritic and perisomatic inhibition in the hippocampal CA1 circuit

Attila Losonczy

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The mammalian hippocampus plays a major role in spatial and episodic learning and memory. Specialized classes of interneurons have evolved for inhibiting dendritic input and perisomatic output domains of the hippocampal excitatory circuit elements during this process. Dendritic and perisomatic inhibitory circuits are selectively impaired in several neuropsychiatric disorders. Despite their critical importance, we lack the basic knowledge of how memory functions are controlled by these specialized inhibitory circuits. In the present talk, I will summarize our recent results on how elementary mechanisms of memory processing are controlled by subcellular domain-specific inhibition in the rodent CA1 hippocampal circuit. I will illustrate that distinct perisomatic and dendrite-targeting inhibitory circuits are regulated by endocannabinoids during this process.

Keywords: dendrite, endocannabinoids, hippocampus, inhibition

Cannabinoid type 1 signaling: the "where" matters

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Despite the complexity of endocannabinoid signaling, the main accepted mediator of the effects of exogenous and endogenous cannabinoids on neuronal transmission is the cannabinoid type-1 (CB1) receptor. This metabotropic receptor is considered as the G protein-coupled receptor expressed at the highest amounts in the brain and its protein levels are comparable to the ones of NMDA and GABA-A ionotropic receptors. However, others' and our recent studies revealed that the nature of the functions of CB1 depends on the cell type where it is expressed. Thus, CB1-dependent regulation of excitatory transmission bears opposite impact onto several brain functions as compared to the control of inhibition. I will present recent data from our group demonstrating such localization-dependent differential functions of CB1, which also suggest that the pharmacological properties of cannabinoid compounds might depend on these parameters.

Keywords: CB1 receptor, knockout, interneuron, GABAergic, glutamatergic

Sex chromosomes direct sex differences

Emilie Rissman

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More than fifty years ago the discovery that manipulation of androgen exposure in neonates affected adult sexual behavior lead to a new paradigm and many subsequent studies of sexual differentiation of brain and behavior. Without a doubt exposure to steroid hormones during development shapes neural development and subsequently behaviors. This paradigm has been used to examine a variety of behaviors and sex differences in a number of parts of the brain. But in healthy fields paradigm shifts are useful and about 15 years ago another mechanism for sexual differentiation was proposed. Simply put the idea was that genes on sex chromosomes might influence sex differences in brain and behavior. Many behavioral and non-behavioral phenotypes are affected by sex chromosome complement including; autoimmune function, pain perception, social behaviors, habit formation, and neural tube development. One behavior we have studied in this regard is male sexual behavior. In typical tests of male sexual behavior when all mice are gonadectomized and treated with testosterone females are faster to mount and thrust and display more of this behavior than males. In this talk I will present data on an X-chromosome gene that we have identified as the causal factor contributing to sex differences in mounting and thrusting behavior in mice. I will also discuss the mechanism of action of this gene.

This work is supported by NIH NS055218.

Sex differences in the brain: an interplay between genes and hormones

Gregor Majdič

Veterinary Faculty, University of Ljubljana and Faculty of Medicine, University of Maribor, Slovenia

Understanding sex differences in brain structure and function are important for understanding many pathological conditions, since many psychiatric and degenerative disorders of the brain have different prevalence between genders. Although it is well established that many sex differences in the brain develop as a consequence of differential exposure to sex steroid hormones during development, several recent studies also suggest a role for sex chromosomes. The influence of sex chromosomes is difficult to separate from sex hormones, since masculinization of the male brain begins in utero, when it is difficult to eliminate the influence of sex steroids secreted by the gonads. We use SF-1 knockout mice that are born without gonads and adrenal glands due to tissue regression prior to their differentiation. These mice are therefore not exposed to endogenous sex steroids and provide a model to study steroid hormone-independent sex differences in the brain. The studies to be presented are focused in two domains: sex differences in gene expression and behavioral studies. The ultimate goal is to understand how sex differences in gene expression result in differential behavior between sexes.

Environment and brain sexual differentiation: what role for endocrine disrupters?

Giancarlo Panzica

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Neuroscience Institute Cavalieri-Ottolenghi (NICO), Orbassano, Italy**

Several environmental substances (synthetic or natural) are able to impact endocrine function (endocrine disrupting chemicals, EDCs) and, therefore, they may have long-term consequences, especially if exposure occurs during embryonic development. Most of EDCs are agonists or antagonists of androgen or estrogen receptors, therefore they may interfere with brain and behavior sexual differentiation.

We present here data collected in our laboratory on two widely used animal models: the mouse and the Japanese quail. In the quail, we investigated the effect of several EDCs [diethylstilbestrol (DES), genistein or ethylene,1,1-dichloro-2,2-bis-p-chlorophenyl (DDE)] administered in eggs on the differentiation of male sexual behavior and of the parvocellular sexually dimorphic vasotocin system. In the mouse we investigated the effects of perinatal exposure to bisphenol A (BPA) or genistein on the sexual differentiation of NO producing system and of the kisspeptin system. We investigated also the organizational effects of these EDCs on sexual, social, and explorative behaviors.

Our data suggest that precocious exposure to EDCs through maternal administration (in mice) or in egg deposition (in quail) may permanently alter some sexually dimorphic circuits and influence in a gender-oriented way some behaviors. In particular, the timing of exposure to EDCs is a critical factor, such that the effects of a particular EDC will vary over the lifecycle of the animal as well as across species and phyla. Therefore, exposure to the estrogenic chemicals during embryonic development has consequences beyond impaired function of the reproductive axis. This makes it very challenging to evaluate the short and long-term effects of EDCs. These compounds are therefore a third player within the nervous system and the evolutionary implications of having them in the normal food supply for certain human populations (i.e. phytoestrogen derivatives from soy), as well as for wild and farm animals should stimulate a wide discussion about their beneficial or adverse role.

Supported by Regione Piemonte (ricerca sanitaria finalizzata), Fondazione San Paolo (Progetto Neuroscienze), Fondazione Cavalieri-Ottolenghi.

Sunday, 25 September, 10:15
[Symposium: Sex differences in brain and behavior]

Sexual differentiation of the human brain: consequences for gender-identity, sexual orientation and neuropsychiatric disorders

Dick Swaab

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Sex differences in the brain are reflected in behavior and in the risk for neuropsychiatric disorders. The fetal brain develops into the male direction during the intrauterine period by a direct action of testosterone on the developing nerve cells, or in the female direction by the absence of a testosterone surge. Since sexual differentiation of the genitals takes place earlier in intrauterine life than sexual differentiation of the brain, these two processes can be influenced independently of each other. Gender identity (the conviction of belonging to the male or female gender), sexual orientation (hetero-, homo- or bi-sexuality), pedophilia, sex differences in cognition and the risks for neuropsychiatric disorders are programmed into our brain structures during early development. There is no proof that the postnatal social environment has a crucial effect on gender identity or sexual orientation. Structural sex differences of hypothalamic nuclei or other brain areas, together with changes of sex hormone levels and their receptors are in close relation to sex differences in behavior and neuropsychiatric disorders. The knowledge of such relationship may help to bring about sex-specific therapeutic strategies.

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Unilateral entorhinal denervation leads to long-lasting dendritic alterations of mouse hippocampal granule cells

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We studied denervation-induced dendritic reorganization of granule cells in the dentate gyrus of the Thy1-GFP mouse. After mechanical transection of the perforant path, single granule cells were 3D-reconstructed at different time points post lesion (3d, 7d, 10d, 30d, 90d and 180 days) and their soma size, total dendritic length, number of dendritic segments and dendritic branch orders were studied. Following entorhinal denervation the granule cell arbor progressively atrophied until 90 d postlesion (reduction of total dendritic length to ~50% of control values). Dendritic alterations occurred in the denervated outer molecular layer, where a loss of distal dendritic segments and a reduction of the mean length of dendritic segments were seen. At 180 d postlesion total dendritic length partially recovered (up to ~70% of control values). This recovery appeared to be the result of a re-elongation of surviving dendrites rather than dendritic re-branching, since the number of dendritic segments did not recover. Thus, the complexity of the granule cell dendritic tree remained permanently reduced. In contrast to the protracted dendritic changes, spine density changes followed a faster time course. In the denervated layer spine densities dropped to ~65% of control values and fully recovered by 30 d post-lesion. We conclude that entorhinal denervation in mouse causes protracted and long-term structural alterations of the granule cell dendritic tree. Spontaneously occurring reinnervation processes, such as the sprouting of surviving afferent fibers, are insufficient to maintain the granule cell dendritic arbor.

Keywords: entorhinal cortex lesion, sprouting, dentate gyrus, regeneration, dendritic spine

siRNA silencing of HIF-1α annulates protecive effect of hypoxia against induced apoptosis of human myoblasts exposed to 1% oxygen under in vitro conditions

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The regeneration process of damaged skeletal muscles is characterised by the activation of satellite cells which are positioned between the basal membrane and the sarcolemma. Activation of these, otherwise quiescent cells, generates a large amount of myoblasts and some of these even under physiological conditions enter apoptosis. When the regeneration environment is changed due to pathological conditions the risk of loosing myogenic precursors increases. Because of its various causes, hypoxia is a frequent condition accompanying the regeneration process. In hypoxia the main cellular adaptation is organized by the hypoxia inducible factor 1α (HIF-1α). HIF-1α controls the expression of a set of genes, some of which promote and some of which inhibit apoptosis. In this study we investigated the influence of HIF-1α on the apoptotic markers in human myoblasts cultured under hypoxic conditions. The level of apoptosis of human myoblasts observed under hypoxic conditions imposed by 1 % oxygen, was relatively low; caspase 3/7 activity was not statistically significantly changed after 2 to 48 hours exposure of myoblasts to such hypoxia. However, when we induced apoptosis of myoblasts by staurosporine, the increase in caspase 3/7 activity in hypoxia was significantly lower ($p \leq 0.001$) than in normoxia. This protective effect of hypoxia was annulated in human myoblasts in which the effects of HIF-1α were blocked by siRNA silencing of its gene. Under such conditions the increase in caspase activity in hypoxia became significantly ($p \leq 0.001$) higher than in normoxia.

Keywords: muscle regeneration, hypoxia, apoptosis

Staurosporine induces apoptosis or primary necrosis in rat astrocytes

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Apoptosis and primary necrosis are highly regulated and interconnected, yet distinct forms of cell death. The distinction between the two forms of cell death is essential as primary necrosis may cause significant cell loss and local inflammation, while apoptosis is essential for tissue homeostasis. Astrocytes are large and diverse cell population in the central nervous system, with many supportive, developmental and protective functions. Therefore, revealing the forms, mechanisms and kinetics of astrocytes death may significantly improve our understanding of pathophysiology of various neurological disorders or mechanical trauma. In this study, cell death was induced in cultured rat astrocytes by staurosporine. Staurosporine is a potent inducer of apoptosis in various cell types including astrocytes; however primary necrosis induced by staurosporine has not been reported yet. Binding of annexin V-FITC and uptake of viability dye 7-amino-actinomycin enabled simultaneous detection and relative quantification of apoptotic and primary necrotic cells by flow cytometry. When low 10-7 M concentration of staurosporine was applied, significantly increased proportion of early apoptotic cells was detected ($p = 0.0005$), in contrast, when high, 10-6 M concentration of staurosporine was applied, significantly increased proportion of primary necrotic cells was detected ($p = 0.000$). Also, both forms of cell death were delayed, as they were only detected after twenty-two hours of regeneration in a staurosporine free medium.

Keywords: rat astrocytes, staurosporine, apoptosis, primary necrosis, flow cytometry

The effect of enriched environment breeding on the perineuronal nets and neurogenesis in tenascin C knockout mice

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In early development tenascin C (TN-C), an extracellular matrix glycoprotein widely expressed in areas of active neurogenesis, forms an integral part of perineuronal nets (PNNs) that play an important role in the regulation of neuronal plasticity. Since it is known that enriched environment conditions (EE) stimulate neurogenesis and neuronal plasticity in normal mice, we investigated the effect of EE on the degree of PNN presence in deep cerebellar nuclei (DCN) and hippocampal neurogenesis in TN-C knockout mice. One group of TN-C knockout mice was bred in EE and the other in standard breeding conditions (SC) for 8 weeks starting from P21. Two groups of wild type (WT) animals of the same strain bred in EE and SC were used as controls. Immunohistochemistry with NeuN for neurons and Wisteria floribunda aglutinin for PNNs in DCN showed no difference in the degree of presence of PNNs between WT and TN-C knockout animals from SC (statistical analysis of the ratio of pixel intensities of both channels in confocal images by two-way ANOVA). However, while difference was apperent between WT animals from SC and EE, there was no such effect of EE on TN-C knockout mice. Further, immunohistochemistry with NeuroTrace (fluorescent neuronal Nissl stain) and double-cortin (neuroblast marker) showed significant increase in hippocampal neurogenesis in WT animals from EE as compared to SC and TN-C knockout animals from EE, while no difference existed between TN-C knockout animals from EE and SC. Therefore, our study emphasizes the primary modulatory role of TN-C in neuronal plasticity.

Keywords: enriched environment, tenascin-C, perineuronal nets

Ischemia-induced neurogenesis in the long-term survival rat model

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Ischemia is a state characterized by the disruption of cerebral blood flow that leads to neuronal dysfunction and cell death. It induces neurogenesis in the subventricular zone (SVZ) containing neural stem and progenitor cells forming neuroblasts. As part of a study on chronic effects of stroke the aim of this research was to explore whether global cerebral ischemia induced neurogenesis in SVZ at the striatal level even up to one year after ischemic insult. Ischemia was induced by chirurgical cardiac arrest lasting 10 min in 2 months old animals (Pluta et al., Acta Neuropathol. 83:1, 1991). For the detection of neurogenesis we used antibodies against doublecortin (DCX) and Ki67 nuclear antigen for the visualization of neuroblasts and proliferating cells, respectively. Labeling neurons was performed using NeuroTrace (fluorescent Nissl stain). Brain sections (30 μm) were analyzed by laser scanning confocal microscopy. Immunostaining with Ki67 and DCX and statistical analysis (unpaired t-test) of the pixel intensities of these markers relative to NeuroTrace showed the presence of significant proliferation of the neuronal progenitor stem cells in the SVZ of ischemic rats as compared to sham controls. Further, in the ischemic rats as opposed to sham controls, an apparent migratory path of DCX-positive cells out of the SVZ was revealed, suggesting that these neuroblasts may still migrate to replace damaged cells in areas affected by neurodegeneration. These findings point to the ongoing neurogenesis in the brain of long-term stroke survivors, and offer a therapeutic target for supporting and promoting functional recovery after stroke.

Keywords: global cerebral ischemia, cardiac arrest, subventricular zone, neurogenesis

Excitatory synaptic input controls the spiking activity of neurons during sharp wave-ripple oscillations in the CA3 region of hippocampal slices

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The CA3 region of the hippocampus can intrinsically generate sharp wave-associated ripple oscillations (SPW-R), synchronous activities that are associated with different cognitive functions. In spite of extensive investigations of SPW-Rs in vivo, the cellular mechanisms underlying the generation of this network phenomenon are largely unknown.

We developed recording conditions under which spontaneously emerging, self-organizing rhythmic activities could be recorded in submerged hippocampal slices resembling SPW-Rs detected in vivo. We first recorded the firing activity of CA3 neurons followed by measuring their synaptic inputs in parallel to monitoring local field potentials. After electrophysiological measurements, we anatomically identified the recorded cells. We observed that pyramidal cells fired action potentials only rarely in conjunction with SPW-Rs (n = 10). Parvalbumin-positive basket cells were the most active neuron type; they readily discharged 4-6 action potentials during a SPW-R (n = 7). Axo-axonic cells fired 1-3 action potentials at the early part of each SPW-R (n = 7). CCK-positive basket cells discharged only occasionally during SPW-Rs (n = 8). Inhibitory cells projecting to the dendritic regions of pyramidal cells (n = 35) fired at low frequencies. Analysis of synaptic currents indicated that the discharge probability and the firing frequency of cells during SPW-Rs correlated with the excitatory, but not with the inhibitory synaptic conductance received by the neurons during these network activities.

Our results suggest that the firing activities of distinct types of neurons during SPW-Rs in hippocampal slices are comparable to those found in vivo. The discharge properties of neurons during SPW-Rs could be, at least partially, determined by their excitatory synaptic input.

Keywords: oscillation, sharp wave-ripple, in vitro

Suppression of excitatory synaptic inputs onto CA3 pyramidal cells and fast spiking basket cells by CB1 cannabinoid receptor activation results in the impairment of hippocampal gamma oscillations

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CB1 cannabinoid receptor activation by exogenous ligands has been shown to impair memory processes that are critically depend on synchronous neuronal activities temporarily structured by oscillations. In this study, we aimed to reveal the mechanisms underlying the cannabinoid-induced decrease of gamma oscillations. Using an in vitro model, we showed that cannabinoid receptor agonists (CP55,940 and WIN55,212-2) suppressed carbachol-induced gamma oscillations in hippocampal slices via activation of CB1 receptors. By simultaneous recordings of local field potentials and firing activity of individual neurons, we found that the cannabinoid-induced decrease in the peak power of oscillations was accompanied by the reduced spiking activity of CA3 pyramidal cells and fast spiking basket cells. Using whole-cell patch-clamp recordings we examined the cannabinoid sensitivity of synaptic input received by neurons in the presence of carbachol. In both CA3 pyramidal cells and fast spiking basket cells, the amplitude of excitatory postsynaptic currents evoked by electrical stimulation was found to be significantly suppressed by cannabinoids. In contrast, evoked inhibitory postsynaptic currents measured in CA3 pyramidal cells were unaltered by cannabinoids. These results together imply that the dampening of cholinergically induced gamma oscillations in hippocampal slices by cannabinoids can be explained by the reduced excitatory input onto CA3 pyramidal cells and fast spiking basket cells caused by CB1 receptor activation, which leads to the less frequent synchronous firing of neurons and thus, to smaller field potentials.

Keywords: gamma oscillation, CB1 receptor

Effect of acute injection of fluoxetine in rats with constitutional upregulation/ downregulation of platelet serotonin transporter

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Serotonin (5-hydroxytryptamine, 5HT) transporter (5HTT) regulates serotonergic neurotransmission by reuptake of serotonin into the presynaptic neurons. 5HTT represents a target molecule for many antidepressants, such as selective serotonin reuptake inhibitors (SSRI), but they do not provide effective treatment outcome for all individuals. Humans with the low expressing form of the 5HTT gene are often resistant to treatment with SSRI. In search for neurochemical basis of the relationship between 5HTT activity and SSRI efficacy we used Wistar-Zagreb 5HT rats, an animal model with constitutionally high or low platelet serotonin level and uptake (termed high- and low-5HT rats), developed by selective breeding toward extremes of these parameters in our laboratory, as a model for studying various aspects of central and peripheral 5HT function.

We examined the acute effect of SSRI, fluoxetine, in Wistar-Zagreb 5HT rats on 5HT-uptake in brain synaptosomes and platelets, as well as platelet and plasma serotonin levels.

Fluoxetine (10 mg/kg, 60 min postinjection) did not cause changes in the activity of central 5HTT (in cortex, hippocampus and raphe nuclei), in either subline of Wistar-Zagreb 5HT rats. In periphery, the acute administration of fluoxetine (1 and 6 mg/kg) resulted in a decrease in platelet 5HTT activity (60 min postinjection) which is differed between the 5HT-sublines. In addition, fluoxetine caused increased plasma serotonin level which was significantly higher in low-5HT animals.

If analogous differences in increase of extracellular 5HT level occur in brain, our model may help to understand differential effect of SSRI on therapeutic outcome.

Keywords: serotonin transporter, fluoxetine, animal model, central and peripheral

Microcystin-LW induces apoptosis of rat cortical astrocytes

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Microcystins (MCs) are a group cyclic heptapeptides produced by several cyanobacteria during cyanobacterial blooms of rivers, lakes and water reservoirs. They are known hepatotoxins, but chronic intoxication affects other organs as well. Although MCs were found in brain tissues of intoxicated animals putative neurotoxic effects are poorly understood. Entry of MCs into a cell is mediated through organic anion transporting peptides, which are expressed in liver, brain and other tissues. This study investigated the effect of MC-LW on rat cortical astrocytes. Astrocytes were plated on poly-l-lysine coated coverslips and incubated with different MC-LW concentrations for 24 hours. Cytotoxicity was monitored by MTT test and apoptotic cells were quantified with annexin V staining. Since MCs are potent inhibitors of cellular protein phosphatases and cause hyperphosphorilation of many cellular proteins, including cytoskeletal elements, cytoskeleton alterations were also studied.

MC-LW induced apoptosis at concentrations higher than 2 micromolar. This effect correlated with reduced absorption in MTT test. Although MC-LW induced apoptosis, there were no changes in cytoskeleton until late stages of apoptosis.

Thus the acquired data suggest that astrocytes are also sensitive to MC-LW. MC-LW induces apoptosis, which is not mediated by cytoskeleton collapse.

Keywords: astrocytes, microcystin, apoptosis

Human anterior lens capsule epithelial cells contraction

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Human anterior lens epithelial cells, attached to surgically isolated capsules, were found to contract upon stimulation. The purpose of this study was to characterize these contractions, which create gaps between cells, and to assess the underlying physiological mechanisms and their possible association with cataract formation.

Lens capsules obtained during cataract surgery were stained with fluorescent dye Fura-2. Its fluorescence, upon excitation at 360 and 380 nm, was imaged to monitor changes in cell morphology and cytosolic free Ca2+ concentrations ([Ca2+]i) in response to pharmacological stimulation by acetylcholine (ACh) and to mechanical stimulation by flow of saline or direct contact.

Epithelial cells contracted in approximately a third of preparations when stimulated by either ACh application, fluid movement or direct mechanical contact. Contractions started either before or at best simultaneously with the rise in [Ca2+]i. Contractions also occurred when there was hardly any change in [Ca2+]i upon application of physiological saline alone. The probabilitiy of contractions occurring did not differ significantly among cortical, nuclear and combined cortical+nuclear cataract.

This study provides the evidence that contractions of the anterior lens epithelial cells take place in significant portion of human lens anterior capsule postoperative preparations after non-specific stimulation. Contractions are at least partially independent of changes in [Ca2+]i. They can be mechanically induced, are localized and reversible and have a fast response and did not differ among different types of cataract. Physiological and clinical significance of this phenomenon remains to be elucidated.

Keywords: cell contraction, lens epithelial cells, mechanical stimulation, intracellular calcium, Fura-2, acetylcholine, cataract

Characteristics of functioning of amygdalar neuronal network during unconditioned fear

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A lot of evidence have been accumulated for the considerable involvement of amygdala in acquisition, expression and extinction of conditioned and unconditioned fear. The data of literature are available on the large involvement of the amygdalar GABAergic system in the reduction and extinction of fear. The aim of this research was to reveal features of amygdalar neuronal network activity during unconditioned fear, manifested as freezing.

Interaction of basal and central nuclear neurons of amygdala was studied during freezing, in a quiet wakefulness and during active orienting exploratory reaction to emotionally significant stimuli. Neuronal activity also was compared under normal conditions and after systemic injection of anxiolytic afobazole (1 mg/kg). Auto- and cross-correlation histograms of neuron spike activities were plotted.

As compared to other states during freezing the number of short-latency (to 100 ms) excitatory connections increased and the number of long-latency (250–450 ms) inhibitory connections decreased, the interaction of neurons was observed with the delta2 range frequencies (2-4 Hz). Afobazole injection resulted in decrease in the probability of freezing after exposure to emotional stimuli. Under the influence of afobazole the duration of the inhibitory interneuronal interactions increased and their latencies changed, the probability of interactions in the delta-range frequency increased, and that in the theta-range decreased. The asymmetry in interhemispheric interactions between amygdalar right-side and left-side neurons with the right dominance decreased. It was concluded that, for the development of fear, the balance between excitatory and inhibitory links of amygdalar neuronal network is essential.

Keywords: amygdala, neuronal interaction, freezing, afobazole, interhemispheric asymmetry

Local synaptic connectivity in the adult auditory cortex

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A current hypothesis suggests that Hebbian-like plastic adaptations of synaptic connections among groups of co-active neurons mediates storage of information in the brain. Understanding how these neuronal assemblies may form it is essential to quantify the underlying synaptic connectivity of these individual neurons. Here, we investigated the excitatory synaptic local circuitry in the auditory cortex. We used coronal brain slices (300 microns) from adult (8-14 weeks old) C57Bl6/J mice and targeted for pyramidal neurons from layers 2/3 and layer 5 for simultaneous quadruple whole-cell patch-clamp. To test for synaptic connections we applied two 5 ms depolarizing current pulses (50 ms apart) in an alternating manner evoking a double action potential in each of these neurons. In a subset of experiments we observed that the presynaptic action potential in one neuron evoked unitary EPSP in the other neuron, indicating synaptic coupling. In layers 2/3, we tested 474 possible synaptic connections and we found 71 functional ones, giving 15% as the overall observed rate of connectivity. In layer 5, this value dropped to 5,1% (17 out of 334 possible connections). In both cortical layers, the connectivity between a given pair of neurons was largely dominated by weak-amplitude connections (mean EPSP amplitude: L2/3 = 0.22 mV; L5 = 0.07 mV) at the expense of rare high-amplitude ones. We find that bi-directional connections in layer 5 are on average larger than those of unidirectional connections. This suggests that in the auditory cortex, similar to the visual cortex, the neuronal network may contain strongly interconnected sub-circuits.

Keywords: auditory cortex, synaptic connectivity, neuronal assemblies

Polyphenols can rescue neurons from necrotic and apoptotic cell death due to oxidative damage

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Many studies have shown that consuming foods of plant origin rich in polyphenols, such as fruits, vegetables, tea and coffee, is associated with reduced incidence of cancer, circulatory and neurodegenerative diseases. Available data suggest that the observed protection can result from polyphenols high antioxidant capacity. Our aims were to check the potential of selected polyphenols that can be found in tea, broccoli, pomegranate and bilberries, to convey neuroprotection from oxidative damage. So, neurons isolated from brain cortex of 18-day-old rat fetuses were cultured for 10 days and incubated for 48 h with 1 and 50 μM of kaempherol (KP), kaempherol glucoside (KPG), epigallocatechin (EGC), epigallocatechin gallate (EGCG) and cynadin-3-glucoside (C3G). In oxidative damage experiments, cells were incubated for 24 h in the presence of polyphenols followed by addition of 150 μM H2O2 and a second 24 h incubation period. Finally, necrotic-like cells death was determined by propidium iodide staining and apoptosis by evaluation of nuclear morphology (Hoechst staining). Results show that all polyphenols, except KPG and EGC higher concentrations, demonstrate significant prevention of H2O2-induced necrotic-like cell death. However, regarding apoptotic cell death, only the highest concentration of KP was unable to abrogate H2O2-induced apoptosis. In both mechanisms, the highest neuroprotection was observed for EGC and EGCG. This suggests different neuroprotection mechanisms that may reside beyond pure antioxidant properties. In conclusion, food polyphenols may constitute a promising preventive approach to neuronal damage but more research must be performed to unravel their mechanistic pathways.

Work funded by FCT, Portugal; Slovene Research Agency

Keywords: polyphenols, necrosis, apoptosis, neuroprotection

Alteration of brain circuits mediating fear and anxiety like behaviors in Steroidogenic factor 1 knockout mice

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Predator odors can be used as stimuli to activate circuits mediating fear or anxiety-like behaviors and therefore are useful for evaluating function under conditions of altered brain development and structure. The transcription factor Steroidogenic factor 1 (SF-1) is required for development of the ventromedial nucleus of hypothalamus (VMH), which is part of the circuitry involved in defensive and anxiety-like behaviors. In the present study to further characterize emotional behaviors in SF-1 knockout (SF-1 KO) mice, the appearance of immunoreactive c-Fos was used as an early marker of neuronal activation in brain regions mediating unconditioned fear responses produced by a cat odor. Immediately after exposure to pieces of cat collars (worn for 3 weeks), mouse brains were processed for immunoreactive c-Fos. SF-1 KO mice spent less time in the vicinity of cat collar than wild type (WT) control mice. Exposure to cat collar increased the number of c-Fos immunoreactive neurons in the dorsomedial part of the VMH ($p < 0.01$) and the posteroventral part of medial amygdala ($p < 0.05$) in WT, but not in the comparable regions in SF-1 KO mice. Analysis of immunoreactive c-Fos suggests that activation of neurons by an unconditioned fear stimulus may be different in SF-1 KO than in WT mice, not only in the VMH which is disorganized in SF-1 KO mice, but also in other brain regions. These studies contribute to our understanding of relationships between brain organization and function in the context of fear responses and fear response circuitry.

Keywords: emotional behaviors, brain, steroidogenic factor 1, c-Fos, cat collar

Distribution and morphology of different GABAergic interneuron subpopulations in the human neocortex

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Gamma-aminobutyric-acidergic (GABAergic) cells form a very heterogeneous population of neurons that play a crucial role in the coordination and integration of cortical functions. Dysfunction or cell death of several specific types has been described as a hallmark of various psychiatric and neurological disorders and it is suggested that organization of GABAergic network shows several features specific for primate neocortex. However, present data are limited and inconsistent, so our aim was to describe, in the human neocortex, distribution and morphology of cortical cells that stain for three main chemical subclasses of GABAergic neurons. Sections of human prefrontal cortex were immunochytochemically stained for parvalbumin (PV), calretinin (CR) and calbindin (CB). PV stained cells have highest density and were diffusely distributed through all cortical layers, frequently with extensive staining of processes. Sublayers of cells with heavy stained cell body were found in supragranular layers and large heavy stained cells were located in the deep part of layer VI. Vast majority of CR positive cells have heavy stained cell body and most of them were located in supragranular layers, only very sporadically in infragranular. Strongly labeled CB positive cells have formed a densely packed belt in the upper part of layer II and in the other layers, both supragranular and infragranular, they were diffusely distributed at lower density. Comparing our data with description in rodents and nonhuman primates, we concluded that organization of cortical GABA-ergic network changed highly through evolution and that even some subtle differences could be seen comparing human and rhesus monkey.

Keywords: interneurons, tangential migration, ganglionic eminence, upper subventricular zone, principal glutamatergic neurons, schizophrenia

Abnormal regulation of the neuron-specific isoform of Elk-1 in response to l-dopa treatment in the 6-OHDA mouse model of Parkinson's disease.

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One of the major complications related to the current pharmacotherapy for Parkinson's disease is the emergence of dystonic and choreic movements following long-term treatment with dopaminergic drugs. These complications are particularly serious in combination with administration of l-dopa, which represent the most effective anti-parkinsonian medication. The 6-OHDA rodent model of Parkinson's disease allows for a biochemical analysis of abnormal cellular pathways associated with the development and persistence of l-dopa induced dyskinesia (LID). Previous research has established hypersensitivity of dopamine D1 receptors and elevated downstream phosphorylation of ERK following administration of l-dopa to 6-OHDA-lesioned rats and mice. It has also been shown that these changes persist during the course of chronic l-dopa administration, leading to the development of dyskinesia. The increase in ERK signaling implicated in LID results in changes in the state of phosphorylation of various downstream target effectors located in the nucleus and implicated in the regulation of gene expression. Here we report elevated levels of the neuronal-specific isoform of Elk1, a transcription factor directly phosphorylated by ERK, in response to both acute and chronic l-dopa treatment. Elk1 has been shown to affect the transcription of various immediate early genes, such as c-fos and zif-268, whose expression is also altered in dyskinesia. Thus, increased phosphorylation of Elk1 may represent an important alteration involved in the development and persistence of LID.

Keywords: l-dopa induced dyskinesia, Parkinson's disease, dopamine receptors, striatum, medium spiny neurons

Structural changes of GABAergic synapses upon fear conditioning in basolateral neurons of the mouse amygdala.

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Previous work showed that Pavlovian fear conditioning induces a dramatic downregulation of benzodiazepine binding sites and transcripts for gephyrin and some GABAA receptor subunits in the basal nucleus of amygdala (BA), which were restored to control levels after fear extinction.

In this work, we analysed by means of the novel freeze-fracture replica immunolabelling technique (SDS-FRL) the synaptic and extrasynaptic content of the GABAA-γ2 subunit in the BA of mice that underwent fear conditioning as well as extinction. Immunogold particles for GABAA subunits tend to concentrate in clusters of intramembrane particles (IMP) on the protoplasmic face of the plasma membrane, indicating that labelled IMP clusters represent GABAergic synapses. The average size of GABAergic synapses in control mice was 0.030±0.019 μm2. Fear conditioned animals showed a significantly ($P < 0.05$, one-way ANOVA) larger (0.041±0.026 μm2) average synaptic size, whereas in mice that underwent extinction it was similar to controls (0.033±0.021 μm2). No differences could be detected in both synaptic and extrasynaptic labelling density for GABAA-γ2, although a clear tendency for a lower density in fear conditioned animals was observed, which however did not reach statistical significance.

We also analyzed by in situ hybridization the mRNA levels of GABAA-γ2 among the 3 groups, which were found highly similar in the BA as well as in other amygdala nuclei. These results suggest that fear conditioning produces an enlargement of GABAergic synapses maintaining the number of GABAA receptors substantially unaltered.

Keywords: amygdala, GABA, fear conditioning, freeze fracture immunolabelling

GAP43 is expressed in early phase of neuronal response on cerebral ischemia

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Growth Associated Protein 43 (GAP43) is related with differentiation of neurons during development, where it is expressed in neuronal growth cones, as well as in activated neurons after an ischemic lesion.

To explore the function of GAP43 in early phase of neuronal response after ischemic lesion we used the transgenic mouse model with luciferase and GFP genes driven by GAP43 promoter. This double reporter system enables a novel in vivo imaging approach using sensitive camera detecting small amounts of light emitted after application of luciferine that is a luciferase substrate. Mice underwent the medial cerebral artery occlusion (MCAO) for 1 hour which corresponds to ishemic lesion in humans.

In vivo imaging has shown that GAP43 is significantly expressed as early as 24h to 7 days after MCAO. To demonstrate expression of GAP43 in challenged neurons that are undergoing cellular stress, we used VivoGlo™ Caspase-3/7 Substrate (Promega) that is a luciferase prosubstrate containing the DEVD tetrapeptide sequence recognized by caspase-3/-7.

The results obtained by double reporter system in the transgenic mice model were verified by immunohistochemistry with GAP43 and GFP antibodies. Colocalisation of GAP43 and NeuN confirmed that activated cells were from the neuronal population. Results showing activation of Caspase-3 and coexpression of GAP43 were supported by colocalisation in double labeling immunohistochemistry with GAP43 and Caspase-3 antibodies.

Considering early onset of GAP43 expression after MCAO and its coexpression with early markers of inflammation and apoptosis it was demonstrated that GAP43 has an additional relevance as a marker of early neuronal stress.

Keywords: GAP43, neuronal stress, MCAO

Distribution profile and quantification of NMDA and mGlu1 receptors within two distinctive dorsolateral pontine nuclei in rats

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Cardiorespiratory coupling, a dynamic property of homeostasis, depends mainly on glutamatergic neurotransmission of the dorsolateral pontine structures, particularly parabrachial (PB) and Kölliker-Fuse (KF) nuclei. In the present study immunohistochemistry was employed to quantify and analize distribution of specific subtypes of NMDA and mGlu1 receptors within PB and KF.

Eighteen adult male Sprague-Dawley rats were perfuse-fixed, their brains frozen and cut into exhaustive series of 40μm-thick sections through the dorsal pons. Immunohistochemistry was performed using polyclonal antibodies against NMDA-NR1, NMDA-NR2A and mGlu1a receptors and appropriate biotinylated secondary antibodies. Labeled neurons in PB and KF were analyzed with light microscope and computer-based image analysis system. Specificity of the labeling was verified by the omission of primary antibodies in adjacent slices from each series.

In both nuclei, NMDA-NR1-immunoreactivity was intense and mainly localized in the neuronal somata with sparse distribution on primary dendrites or extracellular matrix while NR2A-immunoreactivity was generally weak with basically somatical localization. The staining intensity for mGluR1a-immunoreactivity was moderate and observed in both neuronal cytoplasm and extracellular matrix suggesting somatodendritic receptor localization in PB and KF neurons. Quantification of relative receptors levels expressed by integrated optical density (IOD) for PB showed very strong expression of NMDA-NR1 (7.3 ± 0.22), low of NMDA-NR2A (2.2 ± 0.14) and strong-to-moderate of mGluR1a (5.4 ± 0.17). IOD values of NMDA-NR1 (6.7 ± 0.03), NMDA-NR2A (1.8 ± 0.21) and mGluR1a (5.1 ± 0.15) in KF showed similar expression profile.

These results have implications for the organizations of the dorsolateral pontine PB/KF neural circuits that control and modulate cardiorespiratory functions such as blood pressure, heart rate and breathing.

Keywords: glutamate receptors, immunohistochemistry, dorsolateral pons, rats

Morphological and quantitative analysis of neurons in lateral human hypothalamus and distribution of OX1R receptors

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The lateral hypothalamic area (LH) of human hypothalamus is the most prominent part of the lateral hypothalamic zone. The aims of the present study are: (1) to detail the dendritic morphology of Golgi impregnated neurons from the LH and (2) to describe and quantify the dendritic branching features, in an attempt to identify a cytoarchitectonic model for hypothalamic neurons and (3) to describe the OX1R immunoreactivity of lateral hypothalamic neurons. The study is based on 7 hypothalamus' of young individuals who died accidently (Department of Forensic Medicine and Toxicology, Aristotle University of Thessaloniki). The impregnated neurons were analyzed with an image analysis software tool (ImageJ) for the following parameters: number of primary, secondary, tertiary and quartiary dendrites, length of each category of dendrites, total length of dendrites. Finally a Sholl analysis was performed.

Results:

1. The neuronal body protrude 2 or 3 primary dendrites (2-3) which are short in length (39.38±17.69μm).

2. The primary dendrites protrude 4 to 8 secondary dendrites (mean 5.56) which are rather long up to 175 μm (mean length 107.1) and with total length 580.91 ± 270 μm.

3. The total number of dendrites appears to be relatively uniform with mean number 14.16.

4. The Sholl analysis for neurons of Lateral Hypothalamic Nucleus demonstrates a rather uniform pattern of dendritic organization.

5. The large polyhedral neurons of lateral hypothalamic area are strongly immunopositive for OX1R and the receptors are located mainly in the cytoplasm of neurons.

Keywords: morphology, quantitative analysis, lateral hypothalamus, orexin receptors

A novel cell-based fluorescence method of the analysis of BDNF secretion from living neurons

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Brain-derived neurotrophic factor (BDNF) has emerged as a major messenger for activity-dependent brain development and synaptic plasticity. BDNF can be secreted from both axon and soma/dendrites in either mature (mBDNF) or premature (proBDNF) forms. However evidences characterizing spatio-temporal release of different BDNF forms are poor mainly due to absence of appropriate techniques. Here we have developed a new method allowing analysis of BDNF secretion from neuronal cells as well as providing information about the form and processing of secreted BDNF. This approach is based on low percentage transfection of neuronal cells using a bicistronic cDNA plasmid encoding cherry-tagged BDNF (cherry-BDNF) and eGFP as marker of transfected neurons. Cherry-BDNF is released from axons or dendrites of eGFP-positive transfected neurons ("donor" cells), binds to its receptors located on neighbouring non-transfected (eGFP-negative) neuronal and glial cells ("recipient" cells) and thereafter internalized by these target cells. The number of recipient cells as well as the level of cherry-BDNF fluorescence in these cells were reduced in cultures treated with TrkB-IgG, a scavenger of BDNF or with tetrodotoxin, a blocker of neuronal activity. We show also that this approach can be used effectively to detect the form of released BDNF. Obtained evidences indicate high efficacy of proposed approach when used in primary neuronal cultures, organotypic slices or in acute brain slices following in vivo expression.

Keywords: BDNF, trophic factors, neuron

Comparison of electroporation and lipofection for in vitro transfer of plasmid PEGFP-N1 into human myoblasts

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One of main goals of in vitro research is to develop efficient methods for drug delivery. Effective delivery of genetic material into cells is crucial for application in clinical environment for gene therapy or genetic vaccination. The tissue of choice for gene therapy or DNA vaccination are skeletal muscles, representing 40 % of the body mass and which are known to actively participate in the immune response.

Here we compared the efficiency of two different methods for transfer of plasmid DNA: electroporation, where electric pulses are used to permeabilize cell membrane; and lipofection which uses liposomes as carriers. Electroporation is promising for clinical application while lipofection is auspicious for developing strategies for gene therapy. Plasmid pEGFP-N1 coding for green fluorescent protein was transfected into primary human myoblasts with lipofection (Lipofectamine 2000) or by applying high-voltage pulses.

Our results demonstrate that efficient transfection of human myoblasts with DNA in vitro can be obtained both methods. The highest transfection ratios were similar (40.9% with lipofection versus 41.4% with electroporation). However the main difference was in cytotoxicity of both methods. Less than 40% of cells were viable after the electroporation under conditions that give comparable transfection results with lipofection. On the other hand, by lipofection, almost 80% of myoblast were viable after the treatment. Due to lower cytotoxicity, more suitable method for transfection studies on myoblasts in vitro is lipofection. Electroporation has advantage over lipofection in in vivo environment due to simplicity of protocol and lack of need for using additional chemicals.

Keywords: myoblast, lipofection, electroporation, gene therapy

Morphology of astrocytes in subplate in cystic and non-cystic white matter injury of preterm infants

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Despite of the improving medical care, the risk of preterm infants for periventricular white matter injury (PWMI) still prevails. PWMI is represented by a spectrum of injuries ranging from cystic necrotic to non-cystic PWMI. Recent studies suggest that PWMI could also involve subplate (SP) that is situated above WM during development. The aim of this study is to describe the morphology of astrocytes in superficial and deep SP in non-cystic and cystic PWMI compared to controls. We analyzed 29 postmortem human brains diagnosed as cystic and non-cystic PWMI and 10 controls without any neuropathological abnormalities. On paraffin sections, we performed single and double labelings of astrocytes (GFAP, MCT1) and axonal neurofilaments (SMI311). The phenotypic features of astrocytes in SP were analyzed in the frontal lobe. In very preterm infant (24-29 postovulatory weeks, pow) in cystic lesions GFAP astrocytes displayed a non reactive phenotype (small cell bodiy, thin and short processes) and their density was similar to controls. In non-cystic lesions large GFAP positive astrocytes with numerous long processes were found. In preterm infants (30-35 pow) in cystic lesion astrocytes displayed the same features as in cystic lesion of earlier stage. In non-cystic lesion large GFAP astrocytes display peculiar short hairy phenotype in deep SP. The density of GFAP positive astrocytes was significantly increased in deep and superficial SP. This study presents astrocytic phenotypic expressions in SP in non-cystic compared to cystic lesions in PWMI.

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Keywords: subplate, astrocyte

Alteration in perineuronal nets in the somatosensory cortex after photothrombotic stroke in the rats

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Perineuronal nets (PNNs) are a special form of extracellular matrix that envelops the surface of a subset of neurons. The aim of the present study was to examine the alteration of the density and composition of PNNs after focal cortical photothrombotic stroke in rats. The expression of PNNs was studied using Wisteria floribunda agglutinin (WFA) staining and CAT-315 (aggrecan glycoform) and brevican immunohistochemistry at different time points after the insult. In the infarct core, a rapid decrease in WFA-labeled PNN density occurred 1h after the ischemia and almost no WFA-labeled PNNs were found 24 h post-infarct. Some decrease was also observed in the contralateral homotopic area. In perilesional areas 24 h post-infarct, the WFA labeled PNNs as well as the brevican positive PNNs were hardly seen; however, the expression of CAT-315-positive nets was only reduced by 45% as compared to sham control. In prolonged survival times, PNNs tended to reappear, although 30 days after the insult the density of WFA-, CAT-315- and brevican-positive PNNs was still diminished. In remote regions 24 h after the insult, a reduction of WFA-and brevican-positive PNNs occurred, whereas no changes in CAT-315 positive nets were observed. 7 days after the photothrombosis a substantial restoration of PNNs stained with WFA took place, whereas brevican immunoreactivity remained on unchanged level. These results suggest that the PNNs and their components are affected after photothrombosis in a different manner depending on the survival period and the distance from the lesion core.

Keywords: extracellular matrix, brevican, aggrecan, Wisteria floribunda agglutinin, ischemia

The human fetal subventricular zone: regional differences in laminar organization

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The evolutionary expansion of the subventricular zone (SVZ) is a prominent feature of primate brain evolution, reaching its peak in size and complexity in the human fetal telencephalon. In humans, the SVZ appears as the secondary proliferative zone at 7-8 postconceptional weeks (PCW) and produces mostly interneurons, late generated subplate neurons, and glial cells (including oligodendrocyte progenitors). We analyzed the developmental history of the SVZ in human fetuses aged 10 to 28 PCW, by using Nissl and AChE-histochemistry stained frontal and horizontal sections through the telencephalon. At 10 PCW, the SVZ has relatively simple and homogeneous structure, and at 28 PCW it already begins to disappear as a defined architectonic compartment. The SVZ attains the peak of its size and architectonic complexity between 15 and 22 PCW, when it displays a number of sublayers as well as regional differences in the number, arrangement and relative prominence of these sublayers. In addition, there are changes in predominant orientation (tangential vs. radial) of cells in the entire SVZ or its specific compartments, which reflect regional and age-specific differences in the amount and intensity of neuronal migration as well as growth of axonal pathways through specific axon strata. Our findings demonstrate that detailed knowledge on timing and regional differences in SVZ development represents a useful architectonic framework for studying processes of neuronal migration and axonal pathways outgrowth in the human fetal telencephalon using modern molecular biology and gene expression approaches.

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Keywords: telencephalon, cortical interneurons, tangential migration, axon strata

Modulation of glutamatergic synaptic transmission in prefrontal cortex by 5-HT2A receptors

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The serotoninergic system plays an important role in regulating prefrontal cortex functions such as emotional control, cognitive behaviors and working memory. It is also involved in the psychomimetic effects of drugs of abuse and numerous psychiatric diseases. Among the G protein-coupled receptors activated by serotonin (5-HT), 5HT2A receptors raise particular interest. Indeed, they are the target of a large number of psychoactive drugs including atypical antipsychotics (antagonists or inverse agonists) and the majority of psychedelic hallucinogens that act as agonists or partial agonists at 5-HT2A receptors. In parallel, 5-HT2A receptors that are especially abundant in layers IV-V of the prefrontal cortex, with a predominant expression in apical dendrites of pyramidal neurons, have been involved in numerous psychiatric diseases, including psychoses such as schizophrenia. Several studies have shown that activation of 5-HT2A receptors in the prefrontal cortex results in an increase in spontaneous glutamatergic synaptic activity. However, the mechanism of 5-HT2A receptor-mediated modulation of synaptic transmission in prefrontal cortex is still matter of debate. Here, we showed that activation of 5-HT2A receptors modulates synaptic transmission by inducing a depression of AMPA currents on the postsynaptic site and an activation of presynaptic NMDA receptors. Collectively, these data suggest that 5-HT2A receptors exert a pleiotropic action on glutamatergic transmission and may underlie their involvement in spike timing-dependant plasticity.

Keywords: serotonin receptors, prefrontal cortex, synaptic transmission

Synaptic alterations of human caudate nucleus in Alzheimer's disease

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Neostriatum is one of the brain areas that are not primarily affected in Alzheimer's disease, according to classic regard of the disease. However, recent data emphasize the involvement of neostriatum, especially the head of caudate nucleus, in the emergence of characteristic symptoms of the disease.

The present study aims to reveal possible synaptic alterations on medium size spiny neurons of human caudate nucleus in Alzheimer's disease. Postmortem material from the neostriatum of 10 patients suffered from Alzheimer's disease and 10 age-matched healthy individuals were included in the study. The material was obtained from Netherlands Brain Bank and Brain Bank Munich, members of BrainNet Europe. Golgi silver impregnation technique was used for the comparison of the dendritic tree and the spinal density between the two groups. Immunohistochemistry for glutamate receptors (NMDA, AMPA and mGluR) revealed their distribution in the two groups and electron microscopy was used for the final comparison of the synapses in ultrastructural level.

The results show a decrease in spine number and dendritic density of the distal part of the dendritic tree on spiny neurons in Alzheimer's disease. Statistically significant differences were also observed in the distribution of metabotropic glutamate receptors and in the length of synapses in ultrastructural level. These results accomplish previous data about selective degeneration of large cholinergic interneurons in the striatum of Alzheimer's disease patients and reinforce the role of caudate nucleus in the regulation of cognitive functions.

Keywords: striatum, Alzheimer, synapses, glutamatergic receptors, spines

Different distribution pattern and number of proliferating cells along the spinal cord ependyma

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Besides the brain, in the spinal cord, the ependyma of the central canal is thought to be another source of neural progenitor cells. The spinal cord is divided into five regions: cervical, thoracic, lumbar, sacral and coccygeal. Anatomically all these parts are connected via central canal and also with four brain ventricles. Clinical studies report also about existence of ventriculus terminalis - the "fifth ventricle", as a small ependyma-lined cavity in conus medullaris, usually in continuity with the central canal of the rostral spinal cord and associated with glial and neuronal degenerative tissue. Thus, the aim of our study was to compare neurogenic acitivity within the ependyma of conus medullaris and the rest rostral part of the spinal cord. To investigate the proliferation rate in the ependyma of the spinal cord, adult male rats of the first group were injected with a single dose (100 mg/kg) of bromodeoxyuridine (BrdU) and allowed to survive 4h. In the second group of animals, Ki-67 immunohistochemistry was used. Ki-67 is another marker of cell proliferation, which is expressed in dividing cells during the entire division cycle. BrdU and Ki-67 positive cells were counted on 15 randomly selected sections from each spinal cord region. Quantitative analysis showed significantly higher number of proliferating cells in S-phase (BrdU+) in the ependyma of conus medullaris in comparison with the rest spinal cord regions. On the other hand, Ki-67 positivity didn´t exhibit this pattern of distribution and as expected, the Ki-67 method detected significantly more cells than the BrdU method. The results of BrdU immunohistochemistry suggest a privileged status of conus medullaris ependymal lining in adult spinal cord neurogenesis.

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Hemisection of the cervical spinal cord and its effect on descending bulbospinal respiratory pathway

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Spinal cord injury (SCI) often leads to an impairment of the respiratory system, which results in respiratory muscle paresis or paralysis. Respiratory rhythm generation is formed by neurons located in rostral and ventral respiratory group (DRG and VRG). Neurons from medulla project unilaterally or bilaterally down to the phrenic motor neurons located in ventral horn of the cervical spinal cord. Phrenic axons from the spinal cord form the phrenic nerve (PN), which projects to each half of the diaphragm. Nitric oxide/cyclic guanosine monophosphate (NO/cGMP) signaling pathway has become very important in understanding of biological actions expecially in CNS. The best understood trigger for NO/cGMP signalling pathway in CNS is the opening of N-methyl-D-aspartate receptor chanels and the activation of NO synthase (nNOS) in a Ca2+ dependent manner. NO than results in cGMP formation in adjacent neurons through activation of soluble guanylate cyclase (sGC). Because the cGMP is a second messenger for NO inhibition of smooth muscle contraction, we tested the hypothesis that NO acts through cGMP in diaphragm. The crossed neural pathway was revealed using a retrograde tracer Fluorogold. We revealed many FG positive DRG and VRG neurons mostly on contralateral side. Same neurons were nNOS positive. In control, the level of nNOS protein was significantly higher in DRG than in VRG. Spinal cord hemisection followed by 8 days of survival caused significant decrease in the level of nNOS protein in both respiratory groups, but change was more intensive on ipsilateral side. Our experiments show that the crossed premotor bulbospinal pathway contains high number of nNOS-IR fibres terminating in the phrenic nucleus. We confirmed that β1 subunit of sGC is localized in the motor neurons of phrenic nucleus and sGC expression was completely blocked by spinal cord hemisection. We also documented reduction of punctate nNOS-IR around motoneurons of PN after SCI. We performed western blot analysis to determinate the distribution of β1 subunit of sGC in the phrenic nerve. Immunoassay kit was used for analysis of cGMP level in diaphragm. Elisa assay detected a low level of cGMP in diaphragm and unchanged level of sGC after the hemisection in phrenic nerve. The present results indicate that presence of NO activates sGC which also later regulates cGMP.

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Transplantation of neural progenitors after spinal cord injury in the rat

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Spinal cord injury (SCI) often leads to the widespread functional losses of sensory, motor and reflex activity, which are caused by external mechanical forces and subsequential cellular and molecular reactions such as edema, hemorrhage, free radical production, glutamate excitotoxicity, ischemia, the phagocyte response, glial scar formation and apoptosis (of CNS cells population) developed at the center of the lesion. All above mentioned processes significantly contribute to the destruction of gray, white matter and impaired regeneration of the injured tissue. Numerous preclinical experimental treatments use stem cells, which can offer the potential replacement of lost tissue after SCI. Stem cells could specifically promote regeneration through: I) replacement of diseased or dysfunctional cells with healthy, functioning ones, II) production of growth factors, which promote collateral sprouting of damaged axons leading to support of functional recovery. In our study we have analyzed survival, distribution of PKH-67 (fluorescent cell linker dyes) labeled neural progenitors isolated (NPCs) from embryonic rat brain (E16) and their impact on regeneration after SCI. SCI was realized by 2-French Fogarty catheter inserted epidurally at TH8-9 level. After 7 days following SCI, laminectomy was performed and animals were treated with NPCs PKH-67. Optimal dose of cells injection (3µl / 30. 10-3 of cells per injection) was delivered through the glass pipette intraspinally to the lesion site (tip of the pipette aiming 2 mm in the lesion). Seven injections of NPCs were applied to the right and left side of the spinal cord. Animals were sacrificed at 21 days after SCI. Analysis confirmed that implanted NPCs survived two weeks after delivery. In addition, transplanted PKH-67 NPCs were able to migrate and incorporate into the central lesion and fill the cavity. Grafts in damaged tissue could also create appropriate environment enriched with growth factors, which promote outgrowth of damaged axons. In this study we didn't recognize dedifferentiation of transplantation cells into neurons.

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LFS induced LTD of glutamatergic neurotransmission at synapses of rat DRG neurons with rat dorsal horn spinal cord neurons in co-culture

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Primary afferent (DRG) neurons and the dorsal horn spinal cord (DH) neurons play a fundamental role in transmission and modulation of different types of somatic and visceral signalization. We examined the features of induction of use-dependant long-term depression (LTD) of glutamatergic synaptic transmission in DH neurons that was evoked by low-frequency (LFS) 5 Hz conditioning stimulation of DRG neurons. Using dual whole-cell patch clamp recording on the pre- and postsynaptic cells simultaneously we analyzed the excitatory postsynaptic currents (eEPSCs) from DH neurons, evoked by the intracellular stimulation of DRG neurons, in pairs of co-cultivated cells. Monosynaptic eEPSCs were not sensitive to the blockers of NMDA- and/or kainate-receptors (DL-AP5 20 mkM and/or SYM 2081 10 mkM respectively). In co-culture of DRG–DH neurons prolonged conditioning stimulation of DRG cell (5 Hz, 900 pulses, 1 s interval) induced LTD of AMPA-activated eEPSCs amplitudes in DH neurons to 41.6 ± 2.5 % of control at least 20 minutes. For all experiments holding potential at DH neurons was 70 mV. Bath application of the GABAA-receptor antagonist bicuculline (10 mkM) and glycine receptor antagonist strychnine (1 mkM) did not affect LTD. We found out that the LTD magnitude increased with the number of low-frequency conditioning pulses. Consecutive 5 Hz LFS application to DRG neurons with 300, 400, 500, 600 pulses reduced DH eEPSCs amplitudes to 85.6 ± 3.9 %, 62.7 ± 4.3 %, 51.8 ± 3.5 %, 41.6 ± 2.5 % of control respectively. Our data suggest a possibility of induction of activity-dependant homosynaptic LTD of AMPA-receptor mediated glutamatergic neurotransmission at the level of pair synaptically connected DRG and DH neurons in co-culture. Such LFS induced LTD is dependent on timing activation of presynaptic DRG neuron and not requires of postsynaptic DH cell depolarization.

Keywords: long-term depression, low-frequency stimulation, DRG, dorsal horn spinal cord, co-culture, eEPSCs, dual whole-cell patch clamp recording, glutamatergic neurotransmission

Antiretroviral CNS Penetration Effectiveness rank is associated with HIV small fibre neuropathy measured by intraepidermal nerve fibre density

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A method for quantifying penetration of antiretroviral (ARV) drugs was devised and validated in the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) study, providing a rank of combination drug regimens by algorithmically combining the individual drug rankings. The CNS penetration-effectiveness (CPE) rank was associated with CSF VL. Patients treated with regimens having lower CPE ranks showed more residual CSF viral replication than those treated with higher CPE ranks. The present study aimed to investigate whether the CPE rank was also associated with the degree of peripheral sensory polyneuropathy (PSPN).

A total of 102 consecutive HIV patients from an outpatient clinic were studied with clinical examination, electrophysiology, and intraepidermal nerve fiber density (IENFD) for the presence of PSPN. The HIV status, surrogate markers and antiretroviral history was recorded and the CPE rank of the current ARV regimen was calculated. Statistical analysis was executed using SPSS 15.0

Almost 16% presented with symptomatic PSPN and another 36% demonstrated subclinical PSPN, recognized by means of electrophysiology and IENFD determination. IENFD was associated with more advanced HIV disease, lower nadir CD4 count, and exposure to NRTIs. The median CPE rank for the patient population was 1.5 (range 0-3). The CPE rank did not differ in patients with or without PSPN. Using the cutoff value of CPE = 2 the regimen was characterized as CNS effective or not effective. Patients under not CNS effective regimen had lower values of IENFD (3.36±1.75 vs 6.02±2.47, p = 0.02). IENFD correlated with the CPE rank values (r = 0.31, p = 0.045).

The use of ARV therapy capable of penetrating into the CNS was associated with worse IENFD, a measure of small fiber neuropathy, even though patients with and without peripheral sensory polyneuropathy diagnosis did not differ in respect of CPE rank of their ARV regimen.

Audiogenic seizures selectively activate hippocampal neurons in young mice affected by fragile X syndrome

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Patients affected by fragile X syndrome (FXS) present with mental retardation, hyper-reactivity and epilepsy. Fmr1-/- mouse, an animal model of FXS, presents with acoustically induced motor seizures. Our aim was to characterize the contribution of limbic regions to audiogenic seizures (AGS) in Fmr1-/- mice.

AGS susceptibility and immunoreactivity for FosB/ΔFosB, a marker of neuronal activity, were investigated in Fmr1+/+ (wild type, WT) and Fmr1-/- mice 45 and 90 day-old (d.o.).

Unstimulated 45 d.o. Fmr1-/- mice showed higher immunopositivity than unstimulated Fmr1+/+ mice in hippocampus, dentate gyrus and subiculum, suggesting that structures notoriously involved in stress pathways are hyperactive in FXS. Audiogenic test aroused motor responses (wild running -WR-, clonic and/or tonic seizures) in all 45 d.o. Fmr1-/- mice, but not in Fmr1+/+ mice. Of stimulated 90 d.o. Fmr1-/- mice, 25% showed WR behavior, while 75% presented no response. Immunopositivity in hippocampus proper was higher in mice that experienced AGS compared to mice that did not respond to auditory stimulus.

This study i) confirms that susceptibility to AGS is higher in young adult FXS mice compared to age-matched WT and old FXS mice, ii) indicates the acoustic input through the septohippocampal projection as a possible mediator of AGS in FXS.

Evaluation of astrocytomas

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The aim of the study was clinical and imaging evaluation of the astocytomas.

A 32-year-old patient presented to the neurology department with a 25-year history of hemiparesis of the right hand. He did not consult a doctor all these years.

On physical examination, the patient had on oral temperature of 36.6˚C. His pulse was regular with a rate of 68 bpm. His blood pressure was 120/80 mmHg, and his heart sounds were normal. The patient had neurologic deficits in the right side - hemiparesis of the arm and leg, ataxia and atrophy of the right arm. His muscle strenghth was diminished in the right arm and leg, the deep tendon reflexes were exaggerated in right-side and the plantar responses were downgoing bilaterally. These deficits appeared in the 7 year-old, low-grade and he was not investigated. Routine laboratory analysis, incuding a complete blood cell count, a basic metabolic panel and a lipid profile, was normal. Magnetic resonance imaging of the brain with contrast revealed a brain neoplasm - astocytoma (pilocytic).

Low-grade astrocytomas are a heterogeneous group of intrinsic central nervous system neoplasms that share certain similarities in their clinical presentation, radiologic appearance, prognosis and treatment. Improvements in neuroimaging permit the diagnosis of many low-grade astrocytomas that would not have been recognized previously. Low-grade astrocytomas are slow growing and patients survive much longer than those with high-grade gliomas.

People can develop astrocytomas at any age, the low grade type is more often found in children or young adults while the high grade kinds are more prevalent in adults.

Keywords: patient, neoplasm, deficits

Proprioceptive stimulation as a treatment in nystagmus damping

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Recent neuroanatomical and neurophysiological discoveries support the hypothesis that proprioception may be efficient mechanisms for nystagmus damping. The goal of this study is to evaluate the effects of proprioceptive stimulation on the nystagmus suppresion.

15 children with nystagmus were included in this study. They were exposed to the neck-muscle vibration in duration of 25 minutes. The nystagmus waveforms and visual acuity were evaluated prior to and after exposure to the vibrations.

The obtained results suggest that proprioception may be an important factor in the nystagmus damping. It seems that the existence of proprioceptive feedback loops lead to positive changes in the extraocular plant.

Keywords: nystagmus, proprioceptive stimulation, visual acuity

Odor identification and cognitive abilities in Alzheimer's disease

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Research results indicate systemic odor identification deficits in patients with Alzheimer's disease (AD). The aims of this study were: 1) to compare the ability to identify different odors among patients with AD, patients with other type of dementia (OTD) and healthy elderly persons; 2) to compare cognitive status of patients with AD and OTD and 3) to relate the odor identification ability with cognitive functioning for each group, respectively.

The participants were 15 patients with AD, 15 patients with OTD and 30 healthy elderly people, age range 58 to 90. Scandinavian Odor-Identification test was used to assess olfactory function and Dementia Rating Scale (DRS) was used to assess cognitive functions (attention, initiation/perseveration, constructive abilities, reasoning and memory).

The results of one-way ANOVA showed that patients with AD correctly identified significantly less number of presented odors comparing to the patients with other type of dementia and healthy persons. Patients with AD achieved significantly lower scores on initiation/pereseveration and construction subscales comparing to patients with OTD. Finally, significant correlation was found between the olfactory and initiation/perseveration and memory abilties for the patients with AD and the patients with OTD. Interestingly, almost all correlation coefficients among cognitive and olfactory functions were signifant for healthy elderly persons.

According to the results the obvious differences in ability to detect, discriminate and recognize odors among persons with AD and healthy persons exist. The results were discussed in the context of the odor deficit as a characteristic and remarkably sign of Alzheimer's disease.

Keywords: odor identification, Alzheimer's disease, dementia, cognitive abilities

Emotional and temperament profile of remitted patients with major depression and bipolar mood disorder in comparison with healthy volunteers

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Affective disorders have been implicated with temperament and emotional dysregulation. However, there is a scarce of studies dealing with affective temperament in association with emotional regularity as a possible factor for distinguishing between unipolar depression and bipolar disorder.

We aimed to examine the differences in affective temperament and emotional profile between patients with bipolar disorder and unipolar depression in comparison with healthy volunteers.

30 patients with major depression, 21 bipolar outpatients and 28 healthy volunteers were self assessed with TEMPS-A Scale (Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire), and EPI Questionnaire (Emotions Profile Index). TEMPS-A measures five affective temperaments (depressive, cyclothymic, hyperthymic, irritable, anxious), whereas EPI gives an emotional profile with eight emotions arranged in the sense of polarity: protection-destruction, reproduction-deprivation, incorporation-rejection, exploration-orientation.

Our data revealed elevated depressive, cyclothymic and anxious temperament in both clinical groups in comparison with healthy volunteers. Furthermore, bipolar outpatients proved to have less prominent rejection in comparison to depressive outpatients and healthy controls.

Both clinical groups proved to have distinct temperament profile with elevated depressive, cyclothymic and anxious traits, implicating that temperament could indicate a vulnerability to pathological mood regulation. Furthermore, bipolar outpatients presented with less pronounced rejection, showing indecisive personality structure, which could represent a distinct characteristic of bipolar disorder that may potentially separate those patients from patients with unipolar depression. However, further studies are needed to determine whether distinct emotional and temperament profile of bipolar outpatients is associated with dysfunctional neural system for production of affective states, resulting in emotional lability.

Keywords: affective disorders, affective temperament, emotional dysregulation

In vivo differentiation of Richardson's syndrome and progressive supranuclear palsy-parkinsonism from Parkinson's disease: our experience

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Richardson's syndrome (RS) and progressive supranuclear palsy-parkinsonism (PSP-P) are common subtypes of progressive supranuclear palsy (PSP), a cause of atypical parkinsonism in clinical practice. In the majority of cases PSP presents as RS, characterized by postural instability, falls, supranuclear vertical gaze palsy, and cognitive deterioration. A PSP-P is characterized, however, by rigidity, an asymmetric onset, tremor, and moderate initial therapeutic response to L-dopa, thereby rendering differentiation from Parkinson's disease (PD) difficult, particularly during the early course of the disease. Thus, we aimed to identify indicators that distinguish RS from PSP-P in clinical practice.

Twenty-three patients from our outpatient clinic underwent clinical, cognitive, behavioral, speech and CSF (tau, p-tau181) evaluation, as described elsewhere [1]. Additionally, some patients underwent MR imaging.

RS patients showed shorter disease duration and more pronounced neuropsychological and neurobehavioral deficits than PSP-P patients, but did not differ with regard to clinical features and CSF tau. Mid-sagittal and axial T1-weigted images of midbrain showed a marked atrophy and a distinct penguin sign of RS patients, which was not pronounced in PSP-P [2]. On contrary, PD patients showed no abnormality of midbrain tegmentum.

Shorter disease duration of RS patients indicates that RS might have an accelerated disease progression. Recognition of the penguin sign raises suspicion for the diagnosis of RS. A timely and correct diagnosis will result in better targeted treatment strategies and recognition of disease-specific complications.

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Keywords: Richardson syndrome, PSP-parkinsonism, clinical distinction

Proteomic analysis of mouse synaptosomal proteins during development and in a model of Rett syndrome.

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder which is one of the most common causes of mental retardation in females. The most common genetic causes of this disease are mutations in MeCP2 gene which is located on the X chromosome. MeCP2 is a transcriptional regulator and it is believed that the misregulation of downstream targets causes the severe effects associated to RTT. To better understand the molecular basis of RTT disease a global description of affected cells is needed. Our strategy to achieve this goal is a quantitative proteomic approach. We analyzed cortical synaptic proteomes of RTT mouse models in presymptomatic (young) and symptomatic (older) animals and compared them to age matched wild type siblings using iTRAQ labeling technique followed by mass spectrometry. We identified a number of proteins with strongly mis-regulated levels in RTT mice pointing towards specific cellular pathways. We are currently validating these potential targets using independent methods. We hope that our findings may lead to better understanding of the pathology of Rett syndrome that could lead in the future to novel treatment strategies.

Keywords: Rett syndrome, proteomics, iTRAQ

Cerebral and systemic endothelial function in migraine patients

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Cerebral and systemic endothelial function in migraine patients is not well known. It is possible that cerebral endothelial function is altered, especially in the posterior cerebral circulation. Cerebrovascular reactivity (CVR) to L-arginine probably reflects the cerebral endothelium function and in migraine patients has not been determined. In addition, systemic endothelial function in migraine patients, which can be determined by flow mediated vasodilatation (FMD), is also not well known.

Forty migraine patients without comorbidities (20 migraine with (MwA), without aura (MwoA)) and 20 healthy subjects were included. By employing strict inclusion criteria we avoided the possible vascular risk factors. Mean arterial velocity in the middle cerebral artery (MCA) and the posterior cerebral artery (PCA) was measured by transcranial doppler sonography (TCD) before and after infusion of L-arginine, and CVR to L-arginine was then calculated. Systemic endothelial function was measured with FMD.

Migraine patients without cerebrovascular risk factors, both MwA and MwoA, had worse reactivity in PCA ($p = 0.002$). There was not statistically significant difference in reactivity in MCA ($p = 0.29$). Also we did not find statistically significant difference in FMD between migraine patients without cardiovascular risk factors and healthy subjects ($p = 0.96$).

Migraine patients without cardiovascular risk factors have worse endothelial function in the posterior cerebral circulation. It seems that migraine patients without cardiovascular risk factors do not have altered systemic endothelial function. Based on these results it is possible that migraine patients have endothelial dysfunction in the posterior cerebral circulation.

Keywords: migraine, endothelium

Oxidative stress in mild cognitive impairment, a signal for Alzheimer disease?

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Mild cognitive impairment (MCI) is a transitional stage between normal cognitive aging and mild dementia or clinically probable Alzheimer's disease (AD). There is a great interest in the relationship between MCI and the progression to Alzheimer's disease.

The aim of this study was to determine the oxidative stress status in MCI and AD patients.

Mini-Mental State Examination and Alzheimer's Disease Assessment Scale – Cognitive (ADAS-Cog) were used. AD patients fulfilled the NINCDS ADRDA criteria, whereas MCI diagnosis followed the criteria of Petersen. We assessed the levels of some enzymatic antioxidant defences like superoxid dismutase (SOD) and glutathione peroxidase (GPX), as well as lipid oxidation makers like MDA (malondialdehyde). The results were compared to an aged-matched control group.

Alterations in the activity of the antioxidant enzymes were found in MCI and AD peripheral blood compared to age-matched controls. Also, MDA levels were significantly increased in the AD and MCI patients, comparative with the control group. Moreover, a similar decrease of the main enzymatic antioxidant defenses was observed in MCI and AD patients.

It seems that increased production of oxygen reactive species in MCI might lead to rapid consumption of plasma antioxidants. As depleted, the antioxidant systems fail to protect the organism against the oxidative damage. So, in a vicious cycle, MCI and subsequently AD individuals may have an inadequate antioxidant enzymatic activity that is incapable of responding to increased free radical production, which could lead to the development of the pathological alterations that characterize the neurodegenerative disorders.

Keywords: mild cognitive impairment, Alzheimer's disease, oxidative stress

Changes in the EEG spectrum and vegetative indicators while presentation of emotionally significant stimuli in healthy adults, children and patients in a coma

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Patients in a coma or vegetative state, despite of the absence of any external behavioral responses can persist brain activity and emotional and cognitive functions. The level of cognitive functions can be determined by measuring the response of the brain activity to emotionally significant stimuli.

Patients in a coma, healthy adult and children were presented with emotionally significant auditory (cough, growls, laugh, crying, bird song, speech and etc) and tactile stimuli (pleasant, unpleasant, written letters or words and etc). EEG was recorded using portable device "Entsephalan" with polygraph channels.

Presentation of tactile stimuli contributed to changes in the power of theta and delta rhythm in the central and parietal areas. Reduction of the theta rhythm power in the frontal cortex and increased heart rate were observed while listening to baby crying. Increasing the power of the theta rhythm was observed in the occipital region while listening to birdsong. Reducing of the alpha rhythm power was observed in the central and prefrontal regions during presentation of coughing and menacing growls. Changing of theta rhythm power in adult was observed at higher frequencies (4-8 Hz) than in patients in coma and in children (2-6 Hz). These results agree with the data that the alpha rhythm decreased in response to threatening stimuli, and the power of the theta rhythm is associated with affective behavior.

Keywords: EEG, coma, emotional and cognitive functions

A multivariate age adjusted analysis of the effects of anesthetics on the depth of the induced EEG burst suppression pattern

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Functional mapping of the motor cortex (FCM) allows maximal safe lesionectomy. The technique employs electrical stimulation of the eloquent cortex and recording of triggered motor responses in the contralateral muscles. Thus, its results are influenced by anesthetic induced fluctuations of the cortical excitability (CE) and suppression (CS) which require further studying.

The study objective was to determine the impact of anesthetics on the depth of CS, as measured by the lengths of EEG flats in a burst suppression (BS) pattern.

Multivariate linear regression analysis was used to study the effects of propofol, age, halogenated agents and opiates on CE, in 69 patients who underwent FCM, at Massachusetts General Hospital in Boston, MA.

A 50 year old patient anesthetized only with propofol at an infusion rate of 100 mcg/kg/min has an estimated length of the EEG flats of 1.7 seconds ($p < 0.0001$). Every 10 year increase in age above 50, each 10 mcg/kg/min increase in propofol infusion and the use of halogenated agents result in an independent increase in the estimated EEG flats by 0.3, 0.08 and 1.2 seconds respectively (p 0.0005, 0.003 and 0.03).

The depth of BS, as appreciated by the lengths of EEG flats increases linearly with the increase of the propofol infusion rate and is positively correlated with the use of halogenated agents, while nitrous oxide and opiates do not have a significant impact. For the same anesthetic regimen, older patients show a deeper BS pattern.

Keywords: functional cortical mapping, anesthetics, cortical supression

Intraoperative monitoring of S1 nerve-root retraction force and spinal nerve-root potentials during lumbar discectomy – a pilot study

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The correlations between retraction force applied to S1 nerve-root during discectomy, spinal nerve-root potentials (SRPs) amplitude and changes in the postoperative symptoms were investigated.

The pilot study conducted a prospective analysis of 5 patients (3 men and 2 women, mean age 38.8 years ± 9.2 years (range 29–53 years)). A method for simultaneous intraoperative monitoring and measurement of both, retraction force and epidural SRPs amplitude during discectomy was developed [1,2]. Measured data of retraction force and SRPs amplitude were recorded, synchronized and further analyzed. Postoperative neurologic examination was precisely performed regarding worsening of leg symptoms (deterioration of sensory disturbance or pain and motor weakness) three months after operative procedure.

The results of analysis shows that the average force in each discectomy procedure was considerably higher in patients who expressed increased immediate postoperative subjective nerve root symptoms (Pearson correlation $r = -0.95$). There was little association between average pressure in each discectomy procedure and amplitude of SRPs (Pearson correlation $r = 0.3$).

This pilot study suggests that gentle retraction, intermittently released throughout the procedure, and shorter total retraction time may help to minimize nerve root injury during posterior lumbar discectomy.

Keywords: nerve-root retraction force, spinal surgery, intraoperative neuromonitoring, spinal nerve root potential

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Bilateral schizencephaly in a child with congenital cytomegalovirus infection

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Congenital cytomegalovirus (CMV) infection causes multiorgan affection, but the most severe and permanent sequelae are those affecting central nervous system as a result of direct interference of the virus with neurogenesis. We present clinical and neuroimaging results in a 4 month old female patient with CMV infection. Patient is firstborn and mother didn't have any signs of viral infection during pregnancy. During the 25th week of gestation (WG) ventriculomegaly was found by prenatal ultrasound (US), and during 30-32th WG schizencephaly was detected. Results of cordocentesis showed normal XX cariotype. Her BW was 3510g, BL 48cm, Apgar 10/10, head circumference 35cm (85th percentile). Postnatal US confirmed antenatal findings. MSCT showed bilateral schizencephaly with ventriculomegaly and multiple periventricular and parenchymal calcifications, which was clue for diagnosis. Serology on CMV in infant was IgM neg., IgG pos. (3.7 IU/ml, ref. values > 0.4pos.). MSCT findings were later confirmed by MRI/MR-angiography. Repeated findings of serology on CMV in infant was IgM neg., IgG pos. (0.9 IU/ml) and in mother IgM neg., IgG pos. (3.3 IU/ml) while PCR on CMV DNA in serum showed 746 copies/ml and in urine 30500 and repeated > 100000 copies/ml. Patient also has hypoplastic optic nerve and pathological findings of visual evoked potentials as well as dysplasia of right hip. Hearing tests were normal. Currently she receives gancyclovir in therapy. It is important to emphasize that in spite of inconclusive findings of CMV serology and viremia revealed by PCR, key for the diagnosis of congenital CMV were findings of MSCT and high viruria.

Keywords: schizencephaly, congenital cytomegalovirus infection, neuroimaging

Ultrasound in diagnosis of carpal tunnel syndrome

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Carpal tunnel syndrome (CTS) is the most frequent entrapment neuropathy. While the diagnosis is based on clinical parameters, current gold standard for confirmation of CTS in most institutions are electroneurography or electromyography. Recent studies show that some parameters of median nerve on ultrasound can be used in diagnosis of CTS.

An ultrasound device Aloka Alpha10 with a 13MHz transducer and a custom preset was used for imaging. Different parameters of the median nerve (MN) were measured using built-in functions. Cross-sectional area (CSA) of MN was assessed at the inlet and in the middle of the carpal tunnel and flattening-ratio was calculated. Patient group consisted of 20 patients, with bilateral or unilateral CTS symptoms, which were subjected to neurophysiological testing prior to ultrasound examination. Control group had 25 asymptomatic patients that did not undergo examination in electrophysiological lab.

Bilateral symptoms of CTS were present in 75% of patients. Compared to control group, patients with CTS had higher average CSA (9.7 vs. 13.9 mm^2, respectively). By using current standard cut-off value for diagnosing CTS of 10mm^2, sensitivity of 91.2% and specificity of 86.1% was established. EMNG correctly detected 92.8% of symptomatic CTS with specificity of 86.7%. Correlation between semiquantitative values of symptoms and EMG findings was 0.39.

Our findings show comparable sensitivity and specificity of US and EMNG in detection of CTS. By causing no discomfort and possibly revealing visible etiology of CTS, ultrasound could prove to be a convenient method in diagnostic work-up of this condition.

Keywords: CTS, carpal tunnel syndrome, sonography, ultrasound, peripheral nerve

Visual fields in temporal arteritis

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Purpose of the study was to investigate visual field defects in temporal arteritis (TA).

Retrospective study of 49 eyes (35 patients) with TA (69% female; average age 79 years, range 62-92) with positive biopsy (77%, 17/22) or typical clinical picture. Visual fields charted by Goldmann perimeter were categorized as scotomas, peripheral or combined defects. Respect for horizontal meridian was noted.

Loss of vision was unilateral (63%) or bilateral (37%). The most common ischaemic lesions were anterior ischaemic optic neuropathy (AION; 15 unilateral, 4 bilateral) and posterior ischaemic optic neuropathy (PION; 5 unilateral, 5 bilateral). Other lesions included central retinal artery occlusion and internuclear ophthalmoplegia. We observed 8/22 types of visual field defects according to published classification (1). In AION various peripheral defects, which almost universally showed respect for horizontal meridian, (except where only small residual islands remained: 5 eyes) were observed. One eye presented with inferior nasal defect. PION most often presented with scotomas with or without peripheral defect.

Most common visual field defect in AION was peripheral defect with respect for horizontal meridian. Only one eye presented with inferior nasal defect, characteristic for non arteritic AION [1]. PION was most often associated with scotomas combined with peripheral defect.

Reference:

1. Hayreh SS, Zimmerman B. Visual field abnormalities in nonarteritic anterior ischemic optic neuropathy: their pattern and prevalence at initial examination. Arch Ophthalmol. 2005 Nov;123(11):1554-62

Keywords: temporal arteritis, giant cell arteritis, visual field defects, goldmann, kinetic perimetry, ischaemic optic neuropathy, AION, PION

Changes in cognition-related ERPs in early stage sporadic ALS patients

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An increasing need for reliable assessment of cognitive impairments in ALS is not easily met by regular psychometric methods due to their dependence on patients' unimpaired motor function. One of recently considered motor-free alternatives in the assessment of cognitive impairments is the observation of changes in neuro-correlates of cognitive processes such as late event-related potentials (ERPs). The aim of this study was to explore possible electrophysiological markers of cognitive change in non-demented patients with early-stage sporadic ALS using topographical ERP analysis of dense-array EEG recordings.

We recruited 12 patients with sporadic ALS and 11 healthy control subjects. A classical visual two-stimulus "oddball" counting paradigm was used to evoke P3 ERP. Subjects' EEG was recorded with a 128-channel EEG recording system. The Spherical Spline Laplacian (SSL) method was used to estimate average cortical surface potentials in target condition for each subject. A template response was then computed based on the control group grand-average, and both groups were compared on how well they matched the template in ERP amplitude, topography and time-course.

Comparison of patient and control group showed marked differences in all three ERP analysis domains: amplitude, topography and latency. Using binary logistic regression, topography and time-course data provided near perfect prediction of group membership.

Though preliminary, our findings indicate the presence of robust differences in late cognition-related ERPs in non-demented, sporadic ALS patients. Not relying on intact motor function, cognitive ERPs may provide a fruitful alternative to classical psycho-metric methods for testing cognition in ALS patients.

Keywords: event related potentials, P3, amyotrophic lateral sclerosis, cognitive impairment

Cortical activity during conscious and non-conscious breathing

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In humans breathing is mostly a non-conscious automatic motor act, controlled by the brainstem neural networks. However, it can be consciously and voluntarily controlled, e.g. when we perform a brisk inspiratory manoeuvre (sniff) or when we just pay attention to our breathing. During voluntary movements the electrical activity in the sensorimotor areas of the cerebral cortex is changed.

Our aim was to use EEG to study breathing-related rhythmical cortical activity during a) spontaneous non-conscious breathing, b) conscious breathing when the subjects are paying attention to every breath they take and during c) voluntary sniffing. To do this, EEG was recorded together with the airflow in the nostrils and the mechanical changes at the level of thorax and abdomen. The onset of inspiration was used for spliting the EEG into a few seconds-long segments. The wavelet analysis was performed on each segment and then around 100 segments were averaged for each task. The preliminary results show that over the cortical sensorimotor areas the EEG amplitude spectra below 15 Hz are higher during conscious breathing and sniffing than during non-conscious breathing. This provides some insight into cortical control of breathing in healthy individuals and might offer a means for studying cortical control of breathing in patients with respiratory disorders.

Keywords: breathing, sniffing, EEG, wavelet analysis

Impact of fesoteridine treatment of Overactive Bladder (OAB) on brain activation

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In previous functional magnetic resonance imaging (fMRI) studies of patients with Overactive Bladder (OAB), a different pattern of brain activation was shown compared to healthy subjects. The aim of our study was to identify changes of brain activation in patients with OAB using fMRI before and after treatment with antimuscarinic drug fesoteridine.

fMRI was used to measure brain activation related to bladder filling in 5 women with OAB. Before imaging, the bladder was filled with saline to a volume of 100 ml (low volume condition) and 400 ml (high volume condition). During scanning, 50 ml of saline were rapidly infused and withdrawn in 12 s intervals. Scanning was performed at baseline and after 3 months of fesoteridine therapy. Statistical analysis was performed using SPM8. We calculated the difference of brain activation between the high and low volume conditions as well as the difference before and after therapy.

At low bladder volume, there were no significant brain activations neither before nor after therapy. At high bladder volume, significant activations were found bilaterally within the anterior insula, dorsolateral prefrontal cortex, posterior parietal and occipital cortex, as well as in anterior cingulate and supplementary motor cortex. After therapy, brain activity was significantly increased but with a similar distribution to the baseline condition.

Treatment of OAB was related to increased brain activation during bladder filling at higher volume. The significance of this activation change remains to be assessed by comparing the changes to the effect of treatment and to activation patterns in healthy subjects.

Keywords: fMRI, overactive bladder, antimuscarinics

The effects of 40 hours of sleep deprivation and recovery night on circadian profile of human immune cells

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Sleep is considered a restorative process with supportive influences on immune system. Studies showed that sleep deprivation has a negative influence on immune system and health. Disruption of the regular sleep-wake cycle through sleep deprivation may influence the immune system. Our interests were to examine the influence of acute 40 hours of sleep deprivation under light conditions and recovery night on circadian profile of peripheral lymphocyte subsets (CD3, CD4, CD8, CD19) and NK cells.

Blood samples were drawn from nine healthy young volunteers at 4-hour intervals for two consecutive nights, including a night of total sleep deprivation (first night) to determine cell counts of lymphocyte subsets and NK cells. Diurnal variations of cells were tested using analysis of variance (ANOVA). Value of $p < 0.05$ was considered significant. The single cosinor method adapted to a 24-hour period was used for analyzing circadian rhythms of cells. Significant variation was considered if the 95% confidence interval did not include the zero value.

Lymphocyte subsets (CD3, CD4, CD19) and NK cells showed significant circadian variation during the 40 hours of sleep deprivation and recovery night. Cell count peak for CD3, CD4 and CD19 was at night and for NK cells in the morning. In contrast lymphocyte subset CD8 did not show any significant circadian variation during the protocol.

Our study revealed no differences in circadian profile of CD3, CD4, CD8, CD19 lymphocyte subsets and NK cells during the 40 hours of sleep deprivation under light conditions and recovery night.

Keywords: sleep deprivation, circadian profile, human immune cells

Finger-flexion and sniffing related cortical motor potentials in amyotrophic lateral sclerosis – a pilot study

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Cortical motor potential (CMP) is a negative potential preceding the onset of a self-paced movement. It has three components: the Bereitschaftspotential (BP), negative slope (NS) and motor potential (MP). It is generated by the cortical pyramidal cells that are also affected in amyotrophic lateral sclerosis (ALS). Most fMRI studies found increased motor cortical activation in ALS. Two electroencephalographic studies measured lower CMPs in ALS patients with pronounced spasticity and in patients with primary lateral sclerosis.

We hypothesised that the average amplitudes are reduced in ALS due to neuronal loss.

Twenty-one ALS patients and fourteen healthy volunteers were studied. They were assessed for their hand, respiratory and overall functions. EEG was recorded when subjects performed a self-paced sniffing manoeuvre and right finger flexions every 5-10s with 20% of maximal strengths. The average amplitudes of the BP, NS and MP were calculated for the representative central electrodes during both manoeuvres.

Average amplitudes of MPs were significantly higher in ALS patients in most of the representative derivations, while BP and NS average amplitudes did not differ significantly between the groups. There was a strong positive correlation between disease severity (Norris scale scores) and average MP amplitudes during finger-flexion and sniffing.

Contrary to our expectations we found increased amplitudes of CMPs in ALS, presumably as a result of increased cortical excitability documented in such patients. This is compatible with the increased areas of cortical activations found in the majority of fMRI studies.

Keywords: cortical motor potentials, electroencephalography, amyotrophic lateral sclerosis, motor compensation, Bereitschaftspotential

Assessment of autonomic neurotoxicity in 10–16 year old children with different background exposure by heart rate variability

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Heart rate variability (HRV) is used as a marker of the cardiac autonomic function and appears to be promising technique for assessing subclinical effects of lead on the autonomic nervous system. The study group consisted of 60 children (10-16 year old) living in industrial areas (eastern Ukraine). The control group included 87 age-matched healthy children living in recreation areas (Crimea, Ukraine). Lead concentration was measured in hair with the use of X-ray spectrophotometry. Mean lead concentration was 8.99±1.9 micrograms/g in study group and 3.8±0.29 micrograms/g in control ($p < 0.01$ Mann-Whitney U-test). Long-term as well as short-term HRV parameters were taken among all children (n = 147). There was statistically significant increase in TP, VLF and LF in association with an increase in hair lead in the study group ($p < 0.04$). We also observed that higher hair lead was associated with higher RR-interval duration and higher HFnorm and LFnorm among children from control group ($p < 0.04$). This results suggested that lead affects sympathetic activity more strongly than parasympathetic activity.

Keywords: lead, heart rate variability, children

EEG characteristics of men and women with alexithymic personality type

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Severe stressful situations result in alexithymia that is a disorder of emotion cognition constituted by an inability to recognise, interpret, and verbalize physical signs of feelings. The goal of our research was to investigate the EEG characteristics of alexithymic personality type. To determine the level of alexithymia we used TAS test. 82 healthy volunteers (59 women and 23 men) – students aged 18 to 23 years participated in this study, among them alexithymic personality type was found in 19 volunteers. Psychological testing was performed before the EEG registration. EEG was registered over a period of 3 minutes during the rest state. The spectral power density (SPD) of all frequencies from 0.2 to 35 Hz (step 0.2 Hz) was estimated. The Speerman rank test was carried out for the correlation analysis. It was shown that alexithymic personality type in men and women has different EEG characteristics. Namely, we demonstrated SPD decrease in theta2-subband in occipital region and locally in Fz and Cz recording sites as well as in beta1-subband in right anteriofrontal recording site in men. In women we detected SPD depression in alpha3-subband in left occipital region and local desynchronization in beta2-subband in right occipital recording site.

Keywords: alexithymia, EEG

Parameters of ventricular repolarization (QT variability and QTvariability index) in cardiovascular autonomic neuropathy with diabetes patients type 1

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Diabetes mellitus often damages the peripheral nervous system, involving its somatic as well as autonomic part. In cardiovascular autonomic nevropaty (CAN) nerves which innervate heart and blood vessels are affected. The patients with diabetes type I have often CAN and consequently more disturbances in ventricular myocardial repolarization than the diabetic type I patients without CAN.

The aim of our study is to prove ventricular repolarisation abnormalities in diabetic patients type I using new parameters of ventricular repolarisation: QTV and QTVI.

We did a prospective study. 11 diabetic patients type I with CAN, 12 diabetic patients type I without CAN and 33 healthy age matched volunteers participated the study. Their body surface potentials were recorded for 5 minutes with 35 chanel high resolution ECG (HECG). We choose the most apropriate channel and we calculated parameters of ventricular repolarisation (QTV, QTVI).

This study confirmed ventricular repolarisation abnormalities in diabetic patients type I by using new parameters of ventricular repolarisation QTV and QTVI. We found that diabetic patients type I with CAN have ventricular repolarisation abnormalities. We also found ventricular repolarisation abnormalities in diabetic patients type I without CAN (only with QTVI parameter). We conclude that ventricular repolarisation abnormalities in diabetic patients type I without CAN could be caused by structural damage of myocardium or the autonomic neuropathy, that we could not find with clasical autonomic tests.

Keywords: cardiovascular autonomic neuropathy, parameters of heart repolarization, QT variability, QT variability index, diabetes type 1

Glutathione S-transferase gene polymorphisms association with disease severity and progression of multiple sclerosis

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Glutathione S-transferases (GSTs), a multiple gene family of enzymes participating in detoxification processes, may be involved in the pathogenesis of multiple sclerosis (MS). The aim of this study was to investigate relationship of previously estimated glutathione S-transferase P1 (GSTP1) gene polymorphisms with enzyme activity, disease severity/progression, and magnetic resonance (MR) findings in patients with multiple sclerosis (MS). Our previous study showed a distribution of gene GSTP1 polymorphisms A313G (105Ile/Val) and C341T (114Ala/Val) in 58 MS patients compared with age- and gender-related controls. In this study, disease severity and progression were analyzed using Multiple sclerosis severity score (MSSS). Also, the enzymatic activity of glutathione S-transferase was measured in MS patients, found to be carriers of the mutated alleles i.e. genotypes AG/GG (N = 32) and CT/TT (N = 12). In addition, MS patients with higher MSSS score and presence of mutated alleles were examined using conventional MR techniques. Results obtained by MSSS testing showed that patients carrying the GG mutated genotype have significantly higher median MSSS score than patients with AA or AG genotype, while MS patients carrying the TT mutated genotype had the lowest MSSS scores compared with carriers of CC/CT genotype. Slightly decreased activity of glutathione S-transferase was measured in MS patients-mutation carriers compared with mutation non-carriers and controls. We suggest that analyzed gene polymorphisms of GSTP1, involved in antioxidative protection, may contribute to multiple sclerosis pathogenesis. Also, for the purposes of estimation of disease severity, progression and prognosis, utilization of genotype-phenotype analysis proved to be extremely useful.

Keywords: multiple sclerosis, glutathione S-transferase, disease severity and progression

Quality of life outcomes at least 1 year after subarachnoid hemorrhage treatment in Tartu University Clinic

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Aneurysmal subarachnoid hemorrhage (aSAH) is a fatal disease. Many articles have been published about the acute phase of the disease but there is little knowledge about SAH long term outcomes.

We evaluated the quality of life (QoL) in operated aSAH patients and estimated the prevalence of depression and anxiety symptoms.

We studied persons treated surgically for aSAH in Tartu University Hospital over 10 years (2000 to 2010) using Short Form-36 (SF-36) and Emotional State Quetionnaire 2 (ESTQ2). Additionally we collected data about patient comorbid diseases, level of education, employment status and social living situation.

The group of 114 patients studied had significantly lower QoL measured by SF-36 than the general population. The most drastic reductions were in general health, physical role functioning, emotional role functioning, energy and fatigue subscales. Lower QoL and higher depression/anxiety scores were found in those who were older, female, had fewer years of education, and had more time from operation to the interview. Patients had problems with fatigue, irritability, loss of interests, also emotional, sleep and memory disturbances. 30% of the patients had an ESTQ2 score that indicated serious depression and anxiety symptoms, even without any neurological deficit, which is substantially higher than the general population scores. 36% stated that they completely recovered from the disease, QoL scores were significantly higher in this group.

Our results show that a group of surgically treated aSAH patients shows low QoL and high depression/anxiety scores in spite of a good neurological outcome and a substantial amount of time from the operation. At the same time a significant part of the studied group had outcomes comparable to the general population.

Fluoxetine treatment during pregnancy, for better or worse?

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Pregnancy is a period with high-risk for depression, particularly for women with pre-existing psychiatric illnesses. 7%-26% women are depressed during pregnancy. Fluoxetine is the most frequently prescribed drug to battle depression in pregnant women, but its safety in the unborn child has not yet been established. Fluoxetine crosses the placenta, leading to increased extracellular serotonin levels and potentially neurodevelopmental changes in the fetus. So far studies on the consequences of prenatal fluoxetine exposure in humans mainly addressed the potential risk of fetal structural malformations and perinatal complications.

While human studies are hampered by time constraints, rodents offer the possibility to study both the short- and long-term consequences of prenatal fluoxetine exposure. Most studies so far focused on postnatal fluoxetine exposure and behavior later in life. These studies reported increased behavioral despair and anxiety but decreased sexual behavior and aggression. The expression of the serotonin transporter starts at midgestation, suggesting that fluoxetine exposure at an earlier stage of pregnancy in humans or prenatally in rodents will have stronger significant effects on brain development and later life behavior. In this regard, prenatally fluoxetine-treated mice displayed increased anxiety and sensitivity to the rewarding effects of cocaine during adulthood. Thus, evidence has been obtained for effects of prenatal fluoxetine exposure, but the insights are still limited. Therefore we aimed to elucidate the long-term consequences of prenatal fluoxetine in rats. Pregnant rats were injected daily with 12 mg/kg fluoxetine or vehicle from gestational day 11 until birth, and the behavior of the offspring was monitored. Results show that plasma fluoxetine transfer from mother to pup was 83%, and high levels of fluoxetine (13.0 µg/g) were detected in the pup brain 5 hours after the last injection. Prenatal fluoxetine exposure significantly increased anxiety in the novelty-suppressed feeding test, the footshock-induced conditioned place aversion test and the elevated plus maze test (following stress) during adulthood. Prenatal fluoxetine exposed pups displayed significantly decreased components of social play behavior at 4 weeks of age, and a strong tendency for increased self grooming and making less contact in adults. Finally, the hypothermic response to the 5-HT1A agonist flesinoxan was observed at a lower dose in prenatally fluoxetine-exposed rats than in controls.

In conclusion, prenatal fluoxetine exposure in rats leads to detrimental behavioral outcomes in later life, which may partly be due to altered 5-HT1A receptor signaling.

Diagnostic value of cerebrospinal fluid biomarker levels in patients with Alzheimer's disease

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Increased levels of total cerebrospinal fluid (CSF)-tau (here referred to as T-tau) and phosphorylated CSF-tau (P-tau) were found in AD patients.

The aim of our study was to compare CSF levels of beta-amyloid 1-42 (Abeta1-42), total tau (T-tau) and tau phosphorylated at threonine 181 (P-tau181) between AD patients with different age onset of the disease and controls.

We analyzed CSF samples from 98 AD patients (age 74.29 ± 5.41 years) with classic onset of the disease (> 65 years), 35 AD patients (age 54.66 ± 5.38 years) with early onset (< 65 years) and 35 control subjects (age 70.15 ± 11.10 years) using the Innotest (Innogenetics-Belgium) ELISA sandwich tests.

Our results showed (using ANOVA, with age as covariate), that all three biomarkers showed highly significant diagnostic value ($p < 0.001$). Still, no difference was noted among AD patients with classic and early onset of the disease (Abeta1-42 449 ± 209 vs. 460 ± 206 pg/ml; T-tau 697 ± 603 vs. 549 ± 298 pg/ml; P-tau181 130 ± 88 vs. 131 ± 73 pg/ml).

Based on the obtained results, the optimal cut-off values for the previously mentioned biomarkers were calculated. The cut-off value for Abeta1-42 was 563.1 pg/mL, while for both T-tau and P-tau values were determined according to the age groups. In the group under age 65, values determined were P-tau > 83.4 pg/mL, T-tau > 244.7 pg/mL; between ages 65 and 74, values were P-tau > 150.5 pg/mL, T-tau > 541.95 pg/mL; whereas in patients older than 75, cut-off values were P-tau > 146.2 pg/mL and T-tau > 713.2 pg/mL. The cut-off value for Abeta1-42 /P-tau ratio was determined to be < 7.1.

The obtained results suggest that CSF biomarkers may have an important role as supportive diagnostic tool in the diagnosis of AD in routine clinical practice.

Postnatal development of apical oblique dendrites on pyramidal neurons in the human prefrontal cortex

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Our previous study (Petanjek et al. 2008, Cereb Cortex 18:915-929) of large layer IIIC associative cortico-cortical pyramidal neurons (L3N) in the human prefrontal cortex showed that after almost a year-long "dormant" period, these neurons display a unique biphasic developmental pattern of dendritic growth. The second phase of significant elongation appears during the third postnatal year. Analysis of the large layer V cortico-subcortical projecting pyramidal neurons (L5N) showed continuous dendritic elongation during first two postnatal years. This is a typical developmental pattern described in the literature. Since basal and apical dendrites have different source of afferent fibers, we have been interested to see if the side branches of apical dendrites (oblique dendrites) display a similar pattern of dendritic growth. Quantitative analyses of reconstructed neurons impregnated by rapid-Golgi method showed a similar pattern of growth between oblique and basal dendrites. Oblique dendrites of L3N have intensive perinatal dendritic differentiation (up to 3m). After a "dormant" period during infancy, a second large dendritic elongation occurred around the age of 2y. In contrast, dendritic development for L5N appears in a linear pattern within infancy and do not elongate later on. The total length of oblique dendrites was around 3 times lower than the total length of basal dendrites, but the mean length of individual terminal and intermediate segments was roughly the same. We concluded that L3N have a unique pattern of dendritic elongation affecting all parts of dendritic tree, and this elongation takes place during the period of rapid cognitive development in preschool children.

Keywords: associative cortex, cortico-cortical connections neurons, dendritic spines, working memory

Sex differences in early communication development reveal developmental windows for analyzing sex-related differences in early brain maturation

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There are many reports describing sex and gender differences in early language development (e.g., the appearance of the first word; early lexical development). In contrast, there are few data on possible sex differences in early social communication skills. Several studies suggested a significant gender effect on early communication skills; in addition, the prevalence of autistic spectrum disorder (ASD) as the most prominent social communication pathology is significantly higher in boys than in girls. In this study, we used the Communication and Symbolic Behavioral Scales Developmental Profile, Infant-Toddler Checklist (CSBS-DP; Wetherby and Prizant, 2003; translated by M. Cepanec) to collect and analyze data on 1400 girls and boys aged 6 to 24 months. All subjects have been living in family setting in Croatia and had no reported medical disorders. We demonstrated that boys achieved lower scores on CSBS-DP Infant Toddler Checklist, especially with respect to speech and symbolic domain. However, differences in social domain were less prominent. The most significant differences (in all domains) were noticed during two developmental windows: between 13 and 15 and between 20 and 21 months of age. These findings indicate that there may be sex-related differences in brain maturation, especially in language- and cognition-related regions of the cerebral cortex.

Keywords: sex, communication, language, gender

Fronto-parietal role in monitoring predictive visuo-spatial trajectories

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This study investigates whether the monitoring role that has been ascribed to the right lateral prefrontal cortex in various cognitive domains also applies to the spatial domain. Specific questions of the study were (i) what kind of spatial contingencies trigger the putative monitoring function of right lateral prefrontal cortex and (ii) which other brain regions are functionally connected to it in monitoring-related conditions. Participants had to track the trajectory of an imaginary car moving within a roundabout and detect when the car struck the crash-barrier. Four different trajectories were used with different degrees of regularity and predictability, from an almost completely predictable regular trajectory, to a zig-zag one, in which approaching the crash-barrier was not diagnostic of the occurrence of an accident. The findings showed that two regions in the right hemisphere, the lateral prefrontal and inferior parietal cortex, were maximally activated when monitoring regular predictable trajectories as compared with unpredictable ones, demonstrating that this fronto-parietal network plays a role in monitoring environmental contexts that are rich of information about the probability of occurrence of critical events. A psychophysiological interaction analysis showed that the right prefrontal node was functionally connected, not only with inferior parietal lobule, but also with occipito-parietal regions, probably important for tracking the visuo-spatial trajectory, and premotor and sensori-motor regions, probably involved in response preparation, in the predictable vs. unpredictable contrast.

Keywords: monitoring, visuo-spatial tracking, fronto-parietal network

Auditory processing in children with hearing loss associated with otitis media with effusion-gender dependence,ear side effect

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Aim of the study was to investigate influence of small peripheral hearing loss on primary auditory cortical function; possible gender differences and possible further cognitive disturbances.

Prospective study group included 18 female (mean age 7.8 years) and 27 males (mean age 6.5 years) with hearing loss associated with otitis media with effusion. Tonal audiometry for estimation of hearing threshold and speech audiometry sound field discrimination and with earphones for each side of ears respectively performed in all study groups.

Lower frequencies (500Hz, 1000Hz) had higher level of hearing loss than higher frequencies (1000Hz, 2000Hz) ($p = 0.008$) in males and females. Speech discrimination threshold in sound field showed no ear side effect between males and females ($p = 0.169$). Presentation of words through the right ears in females showed lower speech discrimination than right ears in males, while left ears showed equal speech discrimination threshold in both groups. When tested 100% of speech discrimination in males, left ears are more affected than right ears ($p = 0.016$). Females showed no differences between threshold of sound field speech discrimination ($p = 0.891$). When tested emitted sound level for reaching 100% of speech discrimination, there were no differences between right and left ears ($p = 0.799$).

Conductive hearing loss caused lateralization in damage of auditory processing, more pronounced in males than in females. Females have more cognitive disturbances in solving of abstract problems.

Keywords: hearing loss, auditory processing, gender

Cortical connections investigated by magnetic stimulation of the parieto-occipital cortex: a TMS/EEG co-registration study

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Understanding of dynamics and relationships of cortico-cortical connections is fundamental to the comprehension of brain funcionality. In the present study, connectivity activated by stimulation of the parieto-occipital cortex in humans by means of TMS/EEG co-registration has been investigated. 13 healthy subjects were involved in the study. They were comfortably seated, fully relaxed and with closed eyes during 6 experimental sessions. 3 real TMS sessions of about 60 single-pulse TMS stimulations were intermingled and pseudorandomized with 3 sham TMS sessions stimulating the left parieto-occipital region of the brain at 120% of the resting motor threshold. Simultaneously, EEG was acquired and evoked potentials computed.

Source analysis (eLORETA) was used to directly compare real TMS vs sham TMS in a time-window comprised between 11 and 250 ms. Preliminary results suggest significant differences at the start of the window analysis where a strong activation around the point of stimulation was evident in Brodman Area (BA) 7. Moreover, real TMS elicited a significant activation of BAs 6 (medial part), 24 and 32, generally with a bilateral pattern of activation, at about 50 ms after the stimulation. A similar pattern of activation was evident at about 100-115 ms comprising also significant voxels in BAs 8, 31, 9, 33 and 23. Present results are compatible with a pattern of functional connectivity among parieto-occipital and frontal areas involved in complex tasks such as visuomotor or attentive processing.

Keywords: TMS/EEG, parietal cortex, connectivity, eLORETA

Portable BCI device with particular emphasis on signal-to-noise ratio

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A brain-computer interface (BCI) is a device that accepts commands contained in neurophysiologic signals, without using the explicit output pathways of the brain. This can allow paralyzed people to create new communication channels. As a matter of fact, every year thousand of people lose the use of the output pathways of the brain with loss of their ability to communicate. Recent studies, however, have shown that patients can learn to control the amplitude of EEG activity in specific frequency bands over sensorimotor cortex and use it to control an output device. Movement or imagination of movements are accompanied by decrease in mu/beta activity; conversely, rhythm increase occurs in the post-movement period and with relaxation.

We adopted a focal Event Related Desynchronization/Synchronization paradigm of two or more mental states in healthy subjects, elaborated by a specific hardware, to move a cursor on a screen until it touches a predicted target with a user-friendly and economical BCI. We implemented the BCI2000 software as platform for human-BCI in order to extract the feature components of the EEG signals and to use them in CursorTask. The EEG signals were recorded with a MICROMED portable amplifier. Both the screen and the data acquisition device have been interfaced with a laptop computer running BCI2000. We are also developing dry electrodes which, besides avoiding the use of conductive gel, allow the application of electrical filters close to the EEG source with the aim of impairing the signal-to-noise ratio.

Keywords: BCI, sensorimotor cortex, EEG, dry electrodes

Individual-typological differences in human behavior in conditions of the reward choice with risk

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A choice between probability (100, 75, 50, 25, 10, 0%) and valuable reward were investigated in adult. In behavioral experiments the adult put in a situation of a choice between greater, but the risky compensation, and smaller, but received always, make the decision according to situational factors and specific features, such as propensity to risk and care. In a situation of a choice of strategy of behavior the persons were divided in "inclined to risk" and "careful". Individual-psychological differences between groups were the greatest represented in the band of 25% probability of getting a valuable reward. Probability prognosis was more effectively at persons "tended to risk", than at "careful". Under psychological tests "impulsive" people have appeared more inclined to risk, than to cautious. It is shown the synchronism of rhythms EEG an alpha and beta ranges for group of persons tended to cautious in the conditions of 25% of probability of getting a valuable reward.

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Keywords: behavior, choice, probability of reward, risk, care, impulsiveness

Zoning out while reading: what eyes can tell about attention

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While reading a story, a novel, a scientific paper, one can suddenly experience one's mind being somewhere else, occupied with thoughts not related to what was actually read at the moment. The phenomenon of having thoughts unrelated to what one is occupied with at the present is called zoning out or mind wandering. Mind wandering is a common experience that makes up from 30% to 45% of our awake time. Both as a venue of research into consciousness as well as due to its role in performance errors there is an increasing interest in methods of detecting mind wandering. So far mind wandering has been related to physiological measures, such as skin conductance, EEG and fMRI as well as specific behavioural markers. In reading mind wandering has been related to patterns of duration and location of eye fixations, which are however difficult to analyse and track online. The aim of this study was to test the viability of alternative eye-tracking related parameters as markers of mind wandering. So far eye-tracking data was collected from 5 subjects while reading long passages of text combined with random experience sampling and a reading comprehension test. Currently the prediction value and relationship of multiple eye-tracking parameters with experiential reports and comprehension scores are being explored, with specific focus on pupil dilation, vergence and saccade speed parameters, which are easy to track and process in real time. If successful, these methods could contribute to exploration of text comprehension, fluctuations of attention and consciousness.

Keywords: mind wandering, zoning out, attention, reading, text comprehension, consciousness

Alteration of cholinergic transmission and memory functions in the non-transgenic model of sporadic Alzheimer's disease

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Sporadic Alzheimer's disease (sAD) is associated with cognitive deficits and reduced brain expression of cholinergic receptors, development of which is difficult to track in humans. Streptozotocin (STZ, a nitrosourea derivative)-intracerebroventricularly (icv) treated rat represents an experimental sAD model suitable for determining neurochemical and cognitive impairments but cholinergic deficits have been investigated up to 1 month post STZ-icv only.

Adult, male Wistar rats were injected icv with three STZ doses (0.3-3 mg/kg) or vehicle only (controls) and sacrificed three months after the treatment. Cognitive functions were tested by Morris Water Maze Swimming (MWM) and Passive Avoidance (PA) Test before sacrifice. Protein expression of cholinergic muscarinic M1, and nicotinic α7 receptors was measured in the hippocampus (HPC) and parietotemporal cortex (PTC) by SDS-PAGE electrophoresis and immunoblotting. Data were analysed by Kruskal-Wallis and Mann-Whitney U test ($p < 0.05$).

In comparison to the control animals, learning and memory functions in the STZ-icv treated rats were found significantly decreased with 1 and 3 mg dose in both tests (-46.67% and -66.79% by PA; -38.86% and -36.48% by MWM, respectively). One and 3 mg STZ dose significantly altered the expression of muscarinic M1 receptors, manifested as increment in PTC (+82.89% and +67.83%) and decrement in HPC (-18.06% and 15.01%), respectively, while nicotinic α7 receptor expression remained unaltered.

Results show that STZ-icv treatment induces dose-dependent cognitive deficits and cholinergic receptor type-dependent alterations which seem to be also brain region-dependent, suggesting a possible environmental toxin-induced sAD etiopathogenesis.

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Keywords: streptozotocin, sAD, cholinergic receptor

Possible adverse impact of polytherapy on emotionally modulated cognitive control performance in remitted bipolar disorder

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Existent studies report abnormalities in ventral prefrontal cortex which can be observed as pervasive deficits in attention, cognitive control and emotional processing throughout phases of bipolar disorder (BD). Pharmacotherapy treatment for BD has been associated with possible neurotoxic effects on cognition, as some studies suggest an adverse impact of mood stabilisers and antipsychotics on psychomotor speed and aspects of executive functioning. However, none deleterious effects have been reported for the antidepressant treatment in BD.

The study aimed to examine performance on selective attention and emotionally modulated cognitive control tasks in remitted bipolar patients treated with psychotropic medications in different combinations.

Participants were 91 remitted bipolar patients, divided into groups according to their treatment combinations: mood stabiliser (n = 20), mood stabiliser and atypical antipsychotic (n = 15), mood stabiliser and antidepressant (n = 19), atypical antipsychotic and antidepressant (n = 19), polytherapy with mood stabiliser, atypical antipsychotic and antidepressant (n = 18). Computerised Colour-Word Stroop task and the Emotional GoNoGo task were employed.

ANOVA results revealed significant main effects of pharmacotherapy on accuracy and error rates in the Emotional Go/NoGo task. Post hoc analyses showed that the group medicated with mood stabiliser and antidepressant had superior emotionally modulated response inhibition than the group receiving mood stabiliser, atypical antipsychotic and antidepressant.

Our study demonstrates that polytherapy may have more deleterious impact on emotionally modulated cognitive control performance, but not on selective attention in remitted BD. Consistent with current studies, pharmacotherapy with atypical antipsychotics compared to that including antidepressants may indeed have more adverse cognitive profile.

Keywords: bipolar disorder, pharmacotherapy, cognitive control, selective attention, emotional modulation

Patterns of brain rhythms at performing cognitive tasks with gradually changing properties

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This study was designed to investigate the relationships between brain rhythms, mental activity and individual human differences. Our recently obtained results demonstrate that performing mental tasks in mind is accompanied with an establishment of certain rhythmical patterns in subject's EEG. The brain rhythms reflect a mode of thinking performed in mind by a subject, e.g. verbal or spatial thinking. If a task being performed in mind implies using both modes, a mixed rhythmical pattern is observed, which possesses the properties of both. The rhythmical patterns are highly individual, i.e. specific to a particular person, but for a given person they are stable and reproducible, being preserved for at least several months. So, every time a subject performs some certain type of mental activity in mind, a specific rhythmical pattern is established in his/her EEG. To understand how EEG signs of thinking modes change throughout smooth transformation of one mode into another, a line of tasks with gradual changes in spatial and verbal thinking involvement was designed. Specifically, six task types were elaborated, each containing 60 stereotyped tasks presented to 30 healthy subjects while their EEG was recorded. We realized that some tasks required imagination of objects rather than spatial thinking. This mistake led us to an interesting result. We introduced a measure of 'distance' between EEG rhythmical patterns as a statistically calculated index of difference between appropriate power spectra. Thus, quantitative characteristics of EEG rhythmical patterns form a continuum that reflects a continuum of different types of cognitive activity.

Keywords: thinking, brain rhythms, multidimensional scaling, cognitive space

Serotonin enhances cognitive performance: studies on Wistar-Zagreb 5HT rat

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Serotonin (5HT) system plays an important role in processes of learning and memory, especially in consolidation of novel information into long-term memory. Previously, we have developed an animal model, Wistar-Zagreb 5HT rat, with constitutively altered both peripheral and central 5HT homeostasis. Two sublines of the model, developed by selective breeding toward peripheral 5HT parameters, are characterized by increased (high-5HT subline) or decreased (low-5HT subline) values of platelet 5HT level and transporter activity, as well as plasma free 5HT. Sublines also differ in brain 5HT activity, as concluded from pharmacological and behavioral studies.

Different forms of memory were tested in adult males from 5HT-sublines of WZ-5HT rat as follows: spatial memory (assessed in Morris water maze), short-term memory (assessed as novel object recognition) and avoidance conditioning (assessed as step through passive avoidance).

In Morris water maze, high-5HT rats learned platform position faster than low-5HT rats (measured as area under learning curve, $P = 0.02$), but there were no differences in memory score (measured as average distance from previous platform position. Low-5HT rats had tendency to higher novel exploration in novel object recognition task ($P = 0.05$). In the passive avoidance task, 5HT-high rats had higher step through latency ($P = 0.01$) in comparison to low-5HT rats.

Testing of multiple types of memory revealed significant differences between animals from 5HT-sublines, pointing to enhanced cognitive performance in high-5HT animals and confirming the importance of serotonin homeostasis in memory formation. Furthermore, this animal model could represent a valuable tool for further investigation of molecular mechanisms underlying cognitive abilities.

Keywords: cognition, serotonin, memory tests, behavioral analysis, animal model

The comparison of visuospatial working memory in 8- to 12-year-old schoolchildren with and without learning disability

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Learning Disabilities (LD) is one of the most common disorders in schoolchildren, affecting 3 to 10 percent of them. One of the major problems in LD children is working memory (WM) impairments. Since WM has a significant role in reading, writing, mathematical and spelling skills. In this study visuospatial working memory (VSWM) in LD and normal children was compared.

Participants were 142 schoolchildren including 55 LD children (28 males and 27 females) and 87 normal children (51 males and 36 females), all with 8 to 12 years of age. Cards of "overlapping figures" from the "visual perception" subtest of LOTCA test were tachistoscopically exposed to the children for ten milliseconds (0.010 ms) in order for them to point to the pictures they had perceived, on a sheet including the pictures. The results showed that normal children performed significantly better than LDs ($p < 0.001$), normals females gained significantly higher scores than LD females ($p < 0.001$), also normal males significantly higher scores than LD males ($p < 0.001$). No significant difference was found between the two sexes in normal children ($p = 0.386$) but a significant one in LD children ($p < 0.001$) with girls performing better.

LD children of both sexes showed poor visuospatial working memory, suggesting it an underlying factor for LD. No significant difference between the two sexes in the normal group, but a significant one between them in the LD group, with males scoring lower, might suggestively explain higher intensity of LD in boys.

Keywords: working memory, visuospatial working memory, learning disability, children, LOTCA test

A novel method for distinguishing novelty and frequency effect in the modulation of the Nc evoked potential in infants

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Infant recognition memory studies which are of crucial importance for the understanding the development of human memory systems and thereby those systems themselves, lately complemented the extensively used behavioral methods with electrophysiology made possible in very early age. One of the most studied evoked potential component is the "central negativity". The Nc wave shows up on the frontal and central electrodes 400-800 ms after stimulus onset. This component is thought to be related to attentional and memory processes. While theese processes are difficult to tell apart at this early age, it is still a question what is actually modulating the Nc wave. It is interpreted as an attentional orientation response or as novelty detection. To study this question two main paradigms are in use. One uses a visual oddball task where new stimuli are presented in different occurrance probabilities. In the other task a familiarization phase is added, so in the oddball task the children are presented with frequent and infrequent familiar stimuli together with new infrequent ones. One problem of these studies is the difficulty of using an oddball task at this early age and neither of theese studies provided obvious results. In our study we present a new methodology, without using the oddball task, but keeping the three groups of stimuli mentioned above. In addition our method fits with other children memory studies using either familiarization in the lab or stimuli which are already familiar for the children. Thereby providing suitable control for infant recognition memory studies.
(TÁMOP-4.2.1.B-09/1/KMR-2010-0003)

Keywords: infant recognition memory, central negative (Nc) wave

Anatomical, neurochemical and functional consequences of selective cholinergic lesioning combined with local infusion of pre-aggregated amyloid peptide

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Cholinergic loss, senile plaques and tau hyperphosphorylation are important hallmarks of Alzheimer's Disease (AD), however it is yet not known whether and to which extent are interactions between these features required to produce the complex spectrum of pathophysiological changes detected in AD patients. In the present study, the possible existence of functional relationships between the various hallmarks, and their role in producing cognitive impairments have been addressed by combining selective lesioning of the basal forebrain cholinergic neurons with the intrahippocampal injection of pre-aggregated beta (25-35) amyloid peptide, the latter giving rise to local accumulation of oligomers and protofibrils. To this aim, four to five weeks post-surgery, the animals were subjected to sequential testing in several spatial learning and memory maze tasks designed to evaluate reference and working memory abilities, followed by post-mortem morphological and neurochemical assessments. The results show dramatic deficits in both reference and working memory, associated to the occurrence of amyloid aggregates in the neocortex and hippocampus, as well as widespread cholinergic depletions and marked regional increases of APP protein levels, which were more pronounced in the animals subjected to double, but not to either single treatment. The results suggest that the presumed neurotoxicity of the various forms of amyloid requires association with disturbances in monoaminergic (e.g., cholinergic) neurotransmission for inducing cognitive impairments, thus supporting the hypothesis of important functional interactions between these events. Data will also be presented substantiating, in impaired double-lesioned animals, the functional recovery promoted by novel compounds with pro-cognitive and neuroprotective actions.

Keywords: Alzheimer's disease, immunotoxin, amyloid, cognition

Unattended visual change detection: an MEG spatio-temporal source localization study

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Detection of a change in the visual environment is an evolutionary important skill. In auditory modality, mismatch negativity (MMN) response is assumed to reflect an automatic detection of stimulus change. Visual analogue of MMN (vMMN) has also been reported [1] but it is not yet well explored. In this study we used magnetoencephalography (MEG) and spatio-temporal source localization to investigate vMMN response to simple visual stimuli. The evoked visual responses were measured on 10 subjects using Elekta 306-channel MEG system. Gratings of different spatial frequency were presented in an oddball paradigm in three experimental conditions as: 1) frequently-occurring standard stimulus ($P = 0.8$); 2) infrequent deviant stimulus ($P = 0.2$); 3) equiprobable stimulus ($P = 0.2$). The task in all conditions was discrimination of odd/even digits presented in the centre of the gratings. All subjects showed vMMN response to the deviant stimuli in the time interval 100-170 ms. The analysis of the evoked field amplitudes demonstrated differences between the responses to the same stimulus presented as deviant in one condition and equiprobable stimulus in the other condition. Spatio-temporal localization revealed neuromagnetic sources of the vMMN response in the occipital cortex. Our results suggested that unattended visual change was detected by sensory-memory comparison between deviants and standards in the occipital cortex.

Reference:

1. Susac A, Ilmoniemi RJ, Supek S. Sensory memory-based change detection in face stimuli. Translational neuroscience 2010, 1:286-291.

Keywords: MEG (magnetoencephalography), spatio-temporal source localization, vMMN (visual mismatch negativity), change detection, occipital cortex

Early communication development in premature infants: do ex-preterms show autistic profile?

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A number of risk factors can influence the developmental profile of prematurely born infants and children and thus contribute to the pathogenesis of motor and cognitive disabilities. By using Modified Checklist for Autism in Toddlers (M-CHAT), Limperopoulos et al. (2007) reported findings predictive of autism in 26% of ex-preterm toddlers (average age: 22 months). In this study, we used the Communication and Symbolic Behavioral Scales Developmental Profile Infant-Toddler Checklist (CSBS-DP; Wetherby and Prizant, 2003; translated by M. Cepanec) to asses the developmental profile of 50 premature infants and toddlers who were delivered 4 to 10 weaks before the term. We have found that total scores considered to be below the normal values were detected in 16% of prematurely born children. While prematurely born children in general achieved lower scores in all domains (total score, social composite, speech composite, symbolic comosite), a significant difference in comparison to children born at term was found only for the symbolic composite and total score. These findings indicate that prematurely born children display the greatest delay in the cognitive domain, but no primary delay in the development of social communication skills. Thus, the screening scales for autism should be used with great care and one should especiall try to avoid the pitfall of applying it to infants and toddlers of inappropriate (too young) or even unknown developmental age.

Keywords: premature, communication, toddlers, autism

Reduced fear conditioning after AAV-NPY administration into the basolateral amygdala

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Neuropeptide Y (NPY) is a 36 amino acid peptide that is abundantly expressed in the central nervous system. It is involved in various physiological and pathophysiological processes, including energy homeostasis, pain and epilepsy, but also anxiety and depression. Consistent findings have demonstrated an anxiolytic effect of NPY. The presence of different NPY receptors in the amygdala and the effects of NPY on anxiety raise the question, weather, NPY and its receptors may influence acquisition and extinction of conditioned fear. Therefore, we investigated NPY and NPY receptor knockout mice in Pavlovian fear conditioning.

In cued fear conditioning Y1-KO mice show faster conditioning and delayed extinction, whereas Y2-KO mice are similar to wildtype mice. Interestingly Y4-KO mice show normal fear conditioning but impaired extinction. NPY-KO mice acquire higher freezing levels and show increased expression and impaired extinction of conditioned fear.

AAV-Vector mediated re-expression of NPY in the basolateral amygdala (BLA) of NPY-KO mice significantly reduced the increased fear acquisition of NPY-KO mice. In addition extinction was significantly improved after re-expression of NPY in the BLA of NPY-KO mice. No change was observed, however, after re-expression of NPY in the central amygdala.

Our data indicate that NPY has an inhibitory role in the acquisition and facilitates extinction of conditioned fear. These effects seem to be mediated predominantly in the BLA. In particular, the Y1 receptor may modulate the acquisition of fear, whereas for extinction a concerted action of Y1 and Y4 receptors seems to be conceivable.

Keywords: fear conditioning, acquisition, extinction, anxiety, fear

Naringin attenuates D-galactose induced ageing in mice: possible behavioral, biochemical and mitochondrial enzyme alterations

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Role of mitochondrial dysfunction and oxidative stress has been well documented in age related disorders such as Alzheimer's disease. D-galactose has been known to induce aging via the production of oxidants such as reactive oxygen species (ROS), reactive nitrogen species. Bioflavonoids are being used as a neuroprotectants in the treatment of various neurological disorders including aging. Therefore, present study has been conducted in order to explore the possible role of naringin against D-galactose induced cognitive dysfunction, oxidative damage and mitochondrial dysfunction in mice. Chronic administration of D-galactose (100 mg/kg s.c.) for six weeks significantly impaired cognitive task both in both Morris water maze and elevated plus maze, locomotor activity, oxidative defense and impaired mitochondrial complex (I, II and III) enzymes activities as compared to sham group. Six weeks naringin (40 and 80 mg/kg, p.o.) treatment significantly improved behavioral alterations, oxidative defense and restored mitochondria complex enzyme activities as compared to control (D-galactose). Naringin treatment significantly attenuated enhanced acetylcholine esterase enzyme level in D-galactose senescence mice. In conclusion, present study highlights the potential role of naringin against D-galactose induced behavioral, biochemical and mitochondrial dysfunction in mice.

Keywords: ageing, mitochondria, Alzheimer's disease, oxidative stress, neuroprotection

Effect of the categorization task on the N1 visual evoked potential

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In the relevant literature, the visual evoked potential N1 is regarded as the correlate of early perceptual categorization, since it shows clear differences when the subject is shown pictures of objects belonging to different object-categories. We have shown this category effect in our 2009 Sinapsa lecture, together with the effect of color removal which became apparent when form information was obliterated by noise. Careful reading of the literature shows that, with the exception of faces (to which N1 has a special selectivity), all categories were always shown in a task where subjects had to explicitly categorize them (categorization task). Therefore it cannot be said with certainty if the differences in the amplitude of N1 are due to inherent differences to the categories or are somehow elicited by the categorization task itself. In our experiment the subjects categorized the same images in three different conditions, in three different tasks. The images contained two categories (birds and cars) and we made a version of them in which visual noise rendered the categories unrecognizable. The three tasks were two categorization tasks: car vs. bird, chromatic vs. achromatic and a target detection task. We used the target detection task to show the category effects without categorization; the color-discrimination task points out at the category-creating power of the task itself as it enhances the color-difference seen in the case of the visual noise. We analyze the N1 evoked potential to reveal how its characteristics and category-related differences change in function of the given task.

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Keywords: N1 visual evoked potential, categorization, effect of task

Morphological characterization of large intercalated neurons provides novel insight on intrinsic networks of the amygdala

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Although extinction-based therapies are effective treatments for anxiety disorders, the neural bases of fear extinction remains still largely unclear. Recent evidence suggests that the intercalated cell masses of the amygdala (ITCs) are critical structures for fear expression and extinction. They consist of clusters of densely packed medium spiny GABA-ergic neurons surrounding the basolateral amygdaloid complex (BLA). Five percent of ITC neurons are large cells mostly present near the cluster borders. So far, no information is available regarding the neurochemical features, afferents and efferents of large ITC cells, preventing any elucidation of their functional role. We recently discovered that large ITC neurons encircle ITC clusters and display immunoreactivity for either neurokinin 1 or metabotropic glutamate 1α (mGlu1α) receptors. We also found that dendrites of these neurons receive inhibitory inputs from medial capsular projecting- ITC cells. We have recorded and filled with neurobiotin three large mGlu1α positive ITC neurons by means of the juxtacellular technique in vivo. Confocal and pre-embedding electron microscopy demonstrated that these large ITC mGlu1α positive neurons are decorated by axonal terminals enriched in presynaptic mGlu7 and/or mGlu8 receptors. The full tridimensional reconstruction of the in vivo recorded large ITC neurons, combined with immunofluorescence investigations, showed that the axonal arborization widely extends in the rostro-caudal direction and predominantly targets interneurons of the BLA. These findings elucidate for the first time the anatomical features of large ITC neurons and shed new light on intrinsic microcircuits of the amygdala.

Keywords: intercalated cells, amygdala, fear extinction

Antianxiety effect of fluoxetine requires a combination of drug treatment and psychological exposure therapy

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Antidepressants have been used to treat depression and anxiety disorders, including the posttraumatic stress disorder (PTSD), but the mechanism of their action remains unclear. PTSD can be modeled in rodents by Pavlovian fear conditioning. Traumatic conditioned fear memories in mice can be completely erased by extinction during a postnatal critical period, but not in adult animals. We have here investigated whether antidepressant drugs enhance developmental-like neuronal plasticity and reorganize neuronal networks in the brain areas involved in the regulation of fear responses. We have found that in adult male mice chronically treated with fluoxetine either before or after fear conditioning, extinction training induced a permanent loss of conditioned fear memory. In contrast, antidepressant treatment did not reduce fear memory in the mice that were not subjected to extinction training. We found that behavioral changes induced by fluoxetine were associated with altered level of the brain-derived neurotrophic factor (BDNF) in the amygdala and hippocampus, the brain regions critical in the processes of contextual fear memories. Our results suggest that antidepressant fluoxetine can reactivate early-life pattern of conditioned fear memory in adult mice and lead to a long-term erasure of traumatic experiences only when combined with extinction training.

Keywords: fear memory, neuronal plasticity, antidepressants, brain-derived neurotrophic factor

Features of brain asymmetry and situational anxiety depending on self-appraisal

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The aim of current work was investigation of brain mechanisms of situational anxiety depending on self-appraisal level. Self-appraisal level was determined by common methods and tests. Lusher' test was used for estimation of situational anxiety level. According to testing results 30 examinees were divided into 2 groups: with adequate self-appraisal level (ASA) and with high self-appraisal level (HSA). To define functional state of brain hemispheres the ERPs at sites Fp1/2, F3/4 and F7/8 was recorded, and brain asymmetry was estimated by lateralization index (LI) of P3 component amplitude.

Research data showed higher level of anxiety for examinees with adequate self-appraisal than for examinees with high self-appraisal index. At the same time, the greater activity of left frontal and inferior frontal areas for HSA examinees is shown. This data correlates with observed lower anxiety level in this group of examinees, according to functional role of anterior cortical areas in positive emotions' formation and regulation. On the contrary, the greater activity of right frontal and inferior frontal areas for ASA examinees is shown, which correlates with higher level of anxiety. Simultaneously, the greater activity of right orbito-frontal cortex is shown among both groups, however, the greater difference between activities of hemispheres among HAS examinees is revealed.

The greater activity of right orbito-frontal area among HSA possibly predetermines lower level of anxiety, because this area is responsible for positive emotions, as it was shown in our recent studies.

Keywords: brain asymmetry, situational anxiety, self-appraisal

Pilot fMRI study of deployment-ready and novice soldiers mental involvement to presentation of real-life combat videos

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Stressful situations have large impact on our cognition, emotions and behavior. There is a great individual variability in the stress-coping skills which are of vital importance for professionals who work in highly stressful environment. Investigation of neural circuits involved in response to stress may help explain why some individuals are vulnerable and others are stress resilient.

Our pilot fMRI study has been done on a few trained soldiers waiting for deployment in Afghanistan and as many novice soldiers. We used block-design fMRI stimulation paradigm [1] composed of static picture of black "+" sign alternating with three videos of real-life combat clips, with each static picture or video clip lasting 30 s (scanned on 3T Siemens Magnetom TrioTim, 64 x 64 matrix, EPI sequence, TR = 3000 ms; TE = 31 ms; resolution 3.3 x 3.3 x 3 mm). Functional imaging data were processed using SPM 8 software (Wellcome Department of Imaging Neuroscience, London).

Deployment-ready soldiers showed greater bilateral activation in temporo-parietal junction, which is important for negative affectivity and social inhibition, and in the superior parietal lobe, known for integrating different sensory information.

These results suggest that relevance and involvement to stressful combat stimuli is much more evident in deployment-ready soldiers in comparison with novice soldiers.

Reference:

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Keywords: stress, fMRI, soldiers

Bilateral fronto-central EEG synchronization of theta frequencies in verbal and spatial working memory tasks

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Working memory (WM) processes are reflected in synchronized oscillations of theta frequencies (4-8 Hz). The aim of our study was to investigate the pattern of EEG coherence changes in verbal and spatial WM task and the effect of irrelevant speech interference.

Seven healthy volunteers performed two Sternberg-like WM tasks. Participants were presented with a set of letters (V – verbal task) or positions (S – spatial task). They had to maintain their order and later report whether the probe was presented at the indicated position within the set. In interference conditions, irrelevant speech (IS) was presented throughout the tasks (V+IS, S+IS). 128-channel EEG was recorded. Task-related coherences were calculated for all electrode pairs in all frequency bands for maintenance periods and compared between conditions (V, S, V+IS, S+IS). Event-related theta power spectrum changes were calculated using wavelet analysis.

In all participants, a clear, almost identical pattern of increase in theta coherence between fronto-central pairs of electrodes separately in each hemisphere and a decrease of the interhemispheric coherence for V and S tasks was demonstrated. In V+IS condition, there was a decrease in left inferiofrontal theta power during maintenance.

High-density EEG revealed short-range synchronizations within fronto-central regions. The modality-independent increase in fronto-central theta coherence can be interpreted as a reflection of working memory executive function. The effect of irrelevant speech was demonstrated as a decrease in theta power during maintenance in left inferiofrontal region.

Keywords: working memory, EEG coherence, irrelevant speech effect, theta frequency band

Changes in the plasticity of the nervous tissue caused by alterations of the amyloid-degrading enzyme enprilysin expression and activity lead to memory deficit

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The metallopeptidase neprilysin (NEP) has been linked to the pathogenesis of Alzheimer's disease due to its ability to degrade amyloid-β peptide. To study the effects of NEP on neuronal plasticity and behavior we used two paradigms. In the first, adult Wistar male rats (3-4 months) were i.c. injected with a NEP inhibitor phosphoramidon (P, 2.0 mM, 28 days). In the second, adult male rats subjected to prenatal hypoxia (PH, 7% O2, 3 h, E14) which was shown to reduce NEP expression and activity, were i.p. injected with sodium valproate (SV, 200 mg/kg, 24 days) which up-regulates NEP. The data obtained demonstrated that the decrease of NEP activity after P injections or PH led to the disruption of rat memory tested in the radial maze and new object recognition test. On the contrary, injections of SV to the PH animals increased NEP activity and improved their memory. The NEP decrease both after PH and P injections was accompanied by a decrease in the number of synaptopodin-positive labile spines in the molecular layer of the neocortex, while SV injections to HP rats resulted in an increase of this index. Since the number of the labile spines reflects the changes of the neuronal plasticity we suggest, that decreased NEP expression and activity might affect formation of labile axonal-spine contacts in the cortex which can be one of the reasons of cognitive dysfunction after the interventions used in this study.

Supported by RAS "Fundamental Sciences to Medicine", RFBR N10-04-01156.

Keywords: plasticity, memory, prenatal hypoxia, neprilysin, synaptopodin, dendritic spines, rat

Influence of conscious and unconscious thought processes on multidimensional decision making

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According to Unconscious thought theory (UTT), in the case of decisions that involve a choice between a set of multidimensional alternatives, subjects reach better decisions when these are preceeded by periods of inattention to the task than when the decisions are based on conscious deliberation about the advantages and disadvantages of the available alternatives. The aim of the present study was to investigate the effect of available time on the quality of decisions made in conscious thought condition (CTC), and the effect of distraction tasks performed before decision-making in unconscious thought condition (UTC). 72 subjects performed a task that required decision making in UTC and CTC while varying the difficulty of the distraction task in the UTC and the length of available time in the CTC. The results revealed no effect of available time on the quality of decisions in CTC. In comparison to CTC in UTC the participants made significantly worse decisions, especially when the distractor task heavily involved executive functions. The quality of the decisions was however unrelated to distraction tasks performance. Based on the results we conclude that participants have already formed their decisions during the presentation of information and later merely recalled the decision from their working memory. The results do not support UTT as they show that: (i) the quality of decisions is relatively independent from the thought process which occurs in the experimental condition phase, and (ii) a complex distractor task can have a negative influence on the quality of decisions.

Keywords: unconscious thought theory, multidimensional decision making, conscious deliberation, distraction tasks

Electrophysiological correlates of order information coding in visual working memory: preliminary results

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In comparison to identity information, working memory for order information is mediated by different cognitive mechanisms that rely on different neural circuits. There is a lack of studies engaging electrophysiological correlates that would demonstrate what are the differences between item and temporal order coding in visual working memory and in which process those differences occur – in the process of encoding, maintaining or recall.

The aim of our study is to ascertain the specific electrophysiological correlates of order information coding in visual working memory that are distinct from correlates of item coding.

In visual working memory task, the participants are asked to encode and maintain either the identity or temporal order of a set of serially presented visual stimuli while recording EEG signal. The ERP and frequency power associated with encoding, maintenance and recall of items in correct trials of the two task conditions are analysed and compared.

Previous studies demonstrated that theta oscillations are the mechanism by which temporal sequence information is maintained, therefore we expect to see increases in theta power during order memory trials in comparison with item condition. We expect theta power increase during the delay period of serial order trials in comparison with the delay period of item memory trials. In addition, previous studies suggest that frontal and also parietal lobes support temporal order working memory, therefore we expect these effects to be observed at frontal and parietal sites, manifested as power changes in the alpha and gamma bands.

Keywords: visual working memory, temporal order coding, EEG

Extended access to methamphetamine results in lasting cognitive deficits accompanied by decreased surface expression of mGluR2/3 receptors in the rat prefrontal cortex

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Clinical studies have established that chronic abuse of methamphetamine causes persistent neurocognitive and motivational impairments that are paralleled by significant changes in brain metabolism and function, particularly in the frontal cortices and striatum. Amongst animal models, methamphetamine self-administration has the highest face validity, as this model reproduces several key behavioral consequences of methamphetamine addiction in humans. Previously, we have shown that extended access to methamphetamine resulted in escalation of daily drug intake, enhanced drug-seeking, and cognitive deficits in a prefrontal cortex-dependent task. These deficits were accompanied by dysregulated glutamate levels in the prefrontal cortex, with only negligible alterations in tissue dopamine levels. Therefore, the present study characterized methamphetamine-induced changes in presynaptic mGluRs in the prefrontal cortex and striatum. Animals self-administered methamphetamine (0.02 mg i.v. per infusion, or were yoked saline controls) during daily 1-h sessions for 7 days, followed by extended access (6-h) self-administration for another 14 days. After 7-14 days of drug-free abstinence we measured recognition memory in an 'object-in-place' task to assess functional integrity of the prefrontal cortex. One day after testing, the number of functional mGluR2/3 and mGluR7 receptors in the prefrontal cortex and striatum was measured using a surface biotinylation assay. We found that extended methamphetamine self-administration resulted in impaired object-in-place performance and also decreased surface levels of mGluR2/3 in the prefrontal cortex only. Future studies will establish whether a reversal of decreased mGluR2/3 function alleviates methamphetamine-induced behavioral deficits, offering a potential target for therapeutic intervention.

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Keywords: methamphetamine, mGluR, prefrontal cortex, cognitive deficits

The impact of epileptiform EEG discharges on cognitive performance

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Studies of children and adolescents with epilepsy indicate that the presence of epileptiform discharges in the EEG can significantly disrupt cognition. Although it is difficult to deliniate their contribution from other factors that can influence cognition in individuals with epilepsy such as etiology, antiepileptic drugs and seizures, recent experimental data indicates that epileptiform discharges can have a significant transient impact on the speed and accuracy of responding during an experimental task (Kleen et al., 2008).

In our study we investigated the impact of epileptiform EEG discharges on the cognitive performance of 15 children and adolescents with epilepsy and/or epileptiform discharges in the EEG. We analyzed the impact of generalized and focal discharges on the performance of participants on a neuropsychology test battery that included a simple reaction time task, the Test of Test of Variables of Attention (TOVA) and the Computerized Visual Searching Task by (Aldenkamp et al. (1990). The presence of epileptiform discharges was evaluated on a second to second bases by two clinicians, who were blind to the data on cognitive performance. The presence of epileptiform discharges in the EEG was later correlated with cognitive performance data on a case by case basis and group level using mixed linear models. The results indicate a significant impact of epileptiform discharges on the cognitive performance of participants. They also emphasize the need for a comprehensive psychological evaluation of individuals with suspected epileptiform discharges in the EEG.

Keywords: epilepsy, neuropsychology, epileptiform discharges

Noradrenergic contribution to spatial learning and memory: effects of selective lesion and tissue transplants

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Severe loss of noradrenergic neurons in the Locus Coeruleus/SubCoeruleus (LC/SubC) complex and of fiber terminals in the neocortical and hippocampal target regions is known to occur in Alzheimer's disease (AD), however, the exact role played by such transmitter system in the pathophysiology of AD is still unclear. In the present study, the noradrenergic contribution to cognitive functions was investigated following bilateral stereotaxic infusion of the selective noradrenergic immunotoxin anti-dopamine beta-hydroxylase (anti-DBH)-saporin, into the LC/SubC complex of the adult rat. Four to five weeks post-surgery, the animals underwent behavioural tests, administered in sequence, to evaluate reference and working memory abilities, followed by semi-quantitative immunohistochemistry to assess the extent and selectivity of the lesion. When tested in the Morris Water Maze (MWM) task, lesioned animals exhibited only very mild or no deficits in reference memory compared to Normal. By contrast, working memory abilities (assessed by the Radial Arm Water Maze, RAWM task) in these animals were seen significantly impaired, both in terms of latency and errors. Post-mortem morphological analises, carried out on fixed brain tissue, confirmed a massive loss of immunoreactive neurons in the LC/SubC complex, associated to a virtually complete denervation of target areas in the neocortex and hippocampus. The results indicate the existence of a dissociation in the functional effects of the selective lesion, which suggests a role for ascending regulatory noradrenergic afferents in more complex aspects of cognitive performance (i.e. working memory). Data will also be presented concerning the functional effects of implanted progenitors in ameliorating cognitive performance.

Keywords: Alzheimer's disease, noradrenaline, selective lesion, cognition, cell transplantation

Co-expression toggling of microRNAs in Alzheimer's brain

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Alzheimer's disease (AD), mainly characterized by the progressive loss of memory, is an age related complex neurogenerative disorder. According to recent findings, white matter (WM) perturbations also could be important for AD pathology, even though neuropathological lesions (neuritic amyloid plaques and neurofibrillary tangles) are predominantly found in gray matter (GM). In this study, we particularly focus on the expression pattern of microRNAs (miRNAs), a kind of short non-coding regulator RNAs, in GM and WM. MiRNAs have been revealed to be enriched in WM showing differential expression in AD brains. Expression studies with Exiqon Locked Nucleic Acid (LNA) microarrays have been recently carried out separately in cerebral cortical GM and WM of 10 brain samples of elderly females showing early progression of AD to profile a set of miRNAs [1]. We have pursued analyses on this dataset to select the key miRNAs co-expressed differentially in GM and WM, toggling between positive and negative dependence. Through extensive literature survey, we obtained a set of 74 miRNAs reported to be associates with AD. One of these miRNAs already reported to have aberrant expression in AD brains, miR-423-5p, emerged as the hub miRNA in this study. It significantly co-expresses with many AD-associated miRNAs (p-value < 1E–03). It is noted that miRNAs having positive co-expression in WM have higher differential co-expression values than those having positive co-expression in GM. Most interestingly, majority of these miRNAs that are related to AD are enriched in WM.

Reference:

1. W. X. Wang et al., Acta Neuropathology, 121(2):193-205, 2010.

Keywords: Alzheimer's disease, gray matter, white matter, microRNA, differential co-expression

An online brain-machine interface using decoding of movement direction from the human electrocorticogram

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Brain-machine interfaces (BMIs) can be characterized by the approach used to translate brain signals into effector movements. Here we use a "direct motor" BMI approach where movements of an artificial effector (e.g. movement of an arm prosthesis to the right) are controlled by motor cortical signals that control the equivalent movements of the corresponding body part (e.g. arm movement to the right). This approach has been successfully applied in monkeys and humans by accurately extracting parameters of movements from the spiking activity of multiple single-units. We show that the same approach can be realized using brain activity measured directly at the surface of the human cortex (electrocorticogram, ECoG).

Five subjects suffering from intractable pharmaco-resistant epilepsy participated in the study after giving their informed consent. As a part of pre-surgical diagnosis all subjects had 8x8 ECoG grid implants over the hand/arm motor cortex. Subjects interacted with an experimental paradigm shown on a computer screen instructing subjects to move the hand contralateral to the side of the implantation. Subsequently, cursor on the screen was moved according to the movement direction decoded from the subjects' ECoG signals. Significant BMI control was achieved for 4 out of 5 subjects with correct directional decoding in 69%-86% of the trials (76% on average).

Our results demonstrate the principle feasibility of an online direct motor BMI using ECoG signals. Thus, for a direct motor BMI, ECoG might be used in conjunction or as an alternative to the intra-cortical neural signals, with possible advantages due to reduced invasiveness.

Keywords: BMI, ECoG, electrocorticogram

Integration of the inputs to the neo-cortical pyramidal cells and the role of background activity

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Individual neurons in the neocortex exhibit complex dendritic trees, which receive synaptic inputs from various parts of cortical and other brain areas and propagate the inputs to the soma. Many (point) models of neocortical neurons, simplify the effect of the input integration by treating neurons as single points, assuming the inputs to the dendritic tree are linearly summed up in soma. Ignoring the non-linear effects caused by the interaction of the inputs on the dendritic tree and their propagation to the soma, those models miss the potential for computational power of the dendritic tree network (e.g. Poirazi et al., 2003). Using detailed simulations of the neo-cortical pyramidal cells on realistic morphologies we investigate and report on the non-linear effects of the dendritic integration and investigate how these non-linearities are modulated by background input into the neuron, allowing for a state-dependent computation.

Keywords: dendritic integration, single neuron models

Irreversible inhibition of monoamine oxidase B: a computational study

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Monoamine oxidase is a flavoenzyme responsible for the metabolism of important neurotransmitters norepinephrine, serotonin and dopamine. It exists in two isozymic forms, MAO–A and MAO–B. Selective MAO–A inhibitors are used in treatment of depression while selective MAO–B inhibitors are used in the therapy of Parkinson's disease. Clinically used MAO–B inhibitors, selegiline and rasagiline, are irreversible and form a covalent bond with the enzyme flavin adenine dinucleotide (FAD) co-factor upon inhibition. For bond formation associated with irreversible inhibition we assumed acetylenidic mechanism. To investigate the feasibility of this mechanism we performed quantum–chemical calculations in order to estimate the free energy of activation for the reaction between truncated FAD co-factor (flavin moiety) and acetylenide forms of selegiline and rasagiline. On HF/6–31G(d) the calculated free energy of activation for selegiline of 20.2 kcal mol–1 is in very good agreement with the experimental value of 21.3 kcal mol–1. Similar results were obtained for rasagiline, where the calculated value of 21.2 kcal mol–1 [HF/6–31G(d,p) level] is also in very good agreement with the experimentally determined value of 20.8 kcal mol–1. The results of this quantum–chemical study are promising and together with additional experimental and theoretical work they will lead toward deeper understanding of the nature of MAO inhibition and design of novel inhibitors.

Keywords: monoamine oxidase, inhibitors, selegiline, rasagiline, reaction mechanism

Differentiation of parkinsonian and essential tremor using digitalized spirography and a computer decision support system

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Essential tremor (ET) and Parkinson's disease (PD) are among the most prevalent neurological conditions. DATScan can differentiate between ET and PD with specificity of about 97%, but it is a very costly procedure. Digitalized spirography (DS), a method for quantifying tremors, was reported to provide accurate data about tremors. We developed a decision support system (DSS) based on spirography, to help differentiate between ET and PD.

69 patients (31 females) were included into the study; 33 with ET (median age 71.0), 17 with PD tremor (median age 62.5) and 19 with mixed-type tremor (PD and ET characteristics) (median age 70.0). Patients were diagnozed clinically and by using DS. Our DSS was built using argument based machine learning (ABML), which combines machine learning (ML) with expert knowledge.

Differentiating PD and ET with our DSS resulted in accuracy of 80% which shows significant improvement over the usual techniques. During the process of building the DSS, the domain expert was also able to spot his mistake in the initial diagnosis as he overlooked some of the details at the time.

DS, assisted by the use of DSS, can enhance the diagnostic accuracy of tremors while being much cheaper than the DATScan. This approach can be extended to other types of tremor as well. Larger studies and a comparison of the method to the DATScan will help to further define the accuracy of DSS-supported spirography.

Keywords: spirography, argument based machine learning

Fridtjof Nansen (1861–1930): the arctic explorer, the winner of the Nobel peace prize and the co-founder of the neuron theory

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Fridtjof Nansen of Norway is widely known as the Arctic explorer (three-and-a-half-year polar voyage in his ship the Fram), the co-founder of the new science of oceanography, Norway's Ambassador to England, and one of the greatest humanitarians of modern history (he received the Nobel Peace prize in 1922 for his service as the High Commissioner for First World War refugees). However, few people today realize that Nansen started his career as a neuroanatomist and the curator of the Zoological Museum in Bergen, where he completed his doctoral thesis research (1884-1887). He studied the microscopic anatomy of Myzostomida worms as well as the nervous system of anamniote vertebrates. He published an extensive monograph in English and two important articles in German (in Jenaische Zeitschrift für Naturwissenschaften in 1887 and in Anatomischer Anzeiger 1888). He was among the first to learn and apply new Golgi technique (during his 1886 visit to Romeo Fusari in Pavia and subsequent three-month engagement at the Zoological Station in Naples) and the first to employ the Golgi method in the study of invertebrate nervous system. Nansen's work was published simultaneously with papers by Koelliker, His and Forel (1886-1887); all these studies were first to establish the individuality of nerve cells and lay foundation for neuron theory of Ramón y Cajal (1888 onwards). Thus, Nansen should rightly be counted among the founding fathers of the neuron theory. He was also the first to postulate ectodermal origin of Schwann cells.

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Keywords: neuron theory, Golgi method, invertebrate nervous system, fish nervous system

Effect of serum osmolarity changes on cerebrospinal fluid pressure and volume

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We have recently proposed that the cerebrospinal fluid (CSF) volume is under control of osmotic and hydrostatic forces between central nerovus system fluids (blood, interstitial fluid, CSF). In experiments on cats we wanted to investigate the effects of blood osmolarity changes on CSF volume and pressure.

Three types of methods were used on anesthetized cats. The first, ventriculo-cisternal perfusion (12.96 µl/min) before and after i.v. application of 20% mannitol; the second, measuring the outflow of CSF by cisternal free drainage; and the third, measuring CSF pressure in the ventricles of an intact CSF system, with the second and third method being done before and after the i.p. application of a hypoosmolar substance (distilled water).

In the first group, the application of 20% mannitol led to a significantly reduced ($p < 0.005$) outflow volume (from 12.60±0.29 to 0.94±0.09 µL/min). In the second group, the outflow CSF volume significantly increased ($p < 0.001$) after the application of distilled water (from 18.8±0.3 to 28.2±0.7 µL/min). In the third group, after the application of distilled water, the CSF pressure also significantly increased ($p < 0.05$; from 8.3±0.8 to 16.1±0.14 cm H2O).

We conclude that changes in serum osmolarity change the CSF volume because of the osmotic gradient between the blood and all of the CSF compartments, and also that the change in CSF pressure is closely associated with changes in CSF volume.

Keywords: cerebrospinal fluid hydrodynamics, cerebrospinal fluid pressure, cerebrospinal fluid volume, blood osmolarity

High channel count electrophysiology system to investigate thalamocortical interactions

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A novel two dimensional microelectrode array was developed in the framework of the EU-funded research project NeuroProbes. The electrode array comprises CMOS-based (complementary-metal-oxide-semiconductor) integrated circuitry to implement the concept of electronic depth control. The electrode array consists of four 8 mm long slender probe shafts. One shaft contains 513 electrodes in total, placed in two rows on each of the four shafts. The inter electrode distance is 40 micrometer, while the shafts are located 500 micrometers from each other. With the aid of a switch matrix located on the probe shaft, up to 32 recording sites can be selected from the more than 2000 possible electrode channels located on the four shaft probe. The switching electronics implemented on the shafts lets the user to select any combination of two tetrodes on each shaft. This system helps to adjust recording site positions independently inside the brain tissue without any physical movement of the probe.

Acute recording experiments were carried out to test the microelectrode array. Adult rats under ketamine/xylazine anesthesia were used in the experiments. The probes were inserted into the primary somatosensory cortex and the underlying various thalamic nuclei. Good quality local filed potential and multiunit activity was obtained from both the cortex and the thalamus. The analysis of the simultaneously recorded cortical and thalamic signals may give us a powerful tool to reveal more information about the intricate relationship of cortical and thalamic activity.

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Keywords: silicon-based neural probe array, electronic depth control, CMOS integrated circuit, thalamocortical interaction

Cortical plasticity in drug-naive Parkinson´s disease patients

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Drug-naive Parkinson's disease (PD) patients have not been much investigated. But, drug-treated PD patients display number of alterations of motor cortex excitability and plasticity.

Objective of our study was to explain physiological mechanisms of motor cortex between a group of drug-naive PD patients (PDG) and a control group (CG) of healthy subjects.

PDG had 10 patients (3 female), mean age 52.3 years (range 31-75), mean duration of the disease 2.5 years. Hoehn and Yahr stage range was 1 - 2.5 (mean 1.9). CG had 10 subjects (3 female), mean age 50.2 years (range 30-76). Transcranial magnetic stimulation (TMS) elicited motor evoked potentials (MEP), rest motor threshold (rMT), MEP input-output (IO) recruitment curve, silent period (SP), short latency intracortical inhibition (SICI) and intracortical facilitation (ICF) were studied before and after (at 0 and 30 minutes) the paired associative stimulation (PAS) protocol (TMS preceded 25ms by median nerve stimulation), capable of producing long-term potentiation (LPT) like changes.

At baseline PDG and CG did not differ in rMT, MEP size at rest, MEP sizes and SP durations during voluntary contractions and ICF. SICI was significantly weaker in PDG; IO curve had significantly smaller increase in MEP sizes at TMS intensities ≥ 130%. There was no difference in PAS effects between PDG and CG. PAS had no significant effect on other measures.

Results suggest an early deficiency of intracortical inhibitory mechanisms and suggest essentially normal LTP-type plasticity of motor cortex circuits in drug-naive PD patients.

Keywords: drug-naive Parkinson's disease, TMS, plasticity

Developing a deeper understanding of autism through literature mining

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In the field of autism, a complex and heterogeneous developmental disorder, an enormous increase of available information makes it very difficult to connect fragments of knowledge into a more coherent picture. We present a literature mining method, RaJoLink, to search for matched items in unrelated literature that may contribute to a better understanding of the disorder.

214 articles about autism in PubMed database, with their entire text published from 1996 to 2006, served as a source of data. Using ontologies construction (OntoGen) we identified the main concepts of what is already known about autism. Then the RaJoLink (Rare, Joint and Linking) method based on Swanson's ABC model was used to uncover interesting relations.

Among the more interesting concepts identified with RaJoLink in our study were calcineurin and NF-kappaB. Two points of convergence between these agents were showed; the first one is Bcl-2, anti-apoptotic protein, that works mainly at the level of mitochondria and the second is possible immunomodulatory role of both substances. Especially the last one was discussed as potentially important factor regarding the growing evidences about innate and adaptive immune abnormalities in patients with autism.

Further research is needed to give us stronger evidences about calcineurin and NF-kappaB involvement in autism, but in any case, the method could support experts on their way towards discovering hidden relations in data and better understanding of the disorder.

Keywords: autism, literature mining, calcineurin, NF-kappaB

Fractal characterization of surface EMG induced by TMS and peripheral stimulation of the same target muscle

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Since EMG signals demonstrate high complexity, and mechanisms underlying the generation of EMG signals seem to be nonlinear in nature, we investigated how the complexity of surface EMG changes due to TMS stimulation of target muscle (FDI) and when stimulated peripherally for comparison. Our goal was to find whether there would be interaction between the intensity of voluntary contraction and the intensity of TMS stimulation connected with complexity changes. Previous research has shown that fractal dimension is very sensitive factor when describing the subtle changes of surface EMG. RMS was used as linear measure parallel to fractal analysis. Our subjects were six healthy volunteers aged 23 to 45. In both protocols we varied the level of voluntary muscle contraction (mild, moderate and strong) and the intensity of stimulus (both for TMS and current used in peripheral stimulation, we used three levels with respect to threshold stimuli). The analysis was performed over epochs of the surface EMG before the presentation of stimuli, and epochs after induced response (MEP and SP).When compared both linear and fractal measures shoved that complexity of the signal is significantly changing after the application of stimulation. The FD of the EMG after TMS fell in the majority of examined cases (in 42 out of 54 series).

The fall in FD suggests decreased complexity of the voluntary EMG, which is not surprising as it could be expected that TMS would synchronize descending discharges from the motor cortex and spinal motoneuron activity.

Keywords: fractal dimension, surface EMG, complexity changes, TMS

The APASS EEG reference and its utility for EP/ERP applications – theoretical background and preliminary results

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Spatial information obtained by scalp-recorded EEG is limited by the smearing of head current patterns (volume conduction) and by reference electrode effects which cause any recorded potentials to invariably represent activities at two distinct locations. Two general approaches have been developed to overcome these limitations: source modeling (i.e. fitting of equivalent dipoles) and the so-called high-resolutions methods (dura imaging, surface Laplacians). While these methods were proven very useful they often rely upon physiologically unfounded assumptions of source configurations, detailed knowledge of head conduction properties (dura imaging) or are strongly biased towards superficial cortical generators (surface Laplacians). A possible complementary approach would be to estimate reference-free potentials. The use of the average reference in that respect assumes both high electrodes densities and a homogeneous coverage of the entire head surface. While the former condition is often met in modern EEG recordings, the latter never is. We propose the APASS (Absolute Potential Approximation using Surface Splines) reference as a method for improving the estimates of absolute potentials. Splines are used to extrapolate measured potentials beyond the actual electrode array to a more uniform distribution of locations that covers the entire surface of the head. These extrapolated potentials are then used as inputs for the average reference calculation. Analyses of simulated and real EEG data show significant improvements in the estimation of actual EP/ERP amplitudes compared to the classical average reference method. The extent of improvement depends on the specific EEG source configuration that is generating the measured scalp potentials.

Keywords: average reference, reference free potentials, splines, event related potentials, evoked potentials

The effect of cold pressor test on visually evoked cerebral blood flow velocity response

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Tonic pain might have an attenuating effect on the regulation of neurovascular coupling (NVC) in visual cortex, a region unrelated to pain processing. We have investigated this hypothesis by measuring visually evoked cerebral blood flow velocity response (VEFR) before, during and after the cold pressor test (CPT) applied to the hand in 23 healthy human subjects. Transcranial Doppler ultrasound was used for continuous recording of cerebral blood flow velocities (CBFV) in the right posterior cerebral artery (PCA) in three consecutive experimental phases: basal, CPT and recovery. Reversed-pattern checkerboard was used as visual stimulus. VEFR was calculated as the relative increase (%) in CBFV in PCA from the resting (stimulus OFF) to active (stimulus ON) period. During CPT, end-diastolic VEFR increased from 20.2% to 23.6% ($p < 0.05$) and subsequently decreased to 17.7% in the recovery phase ($p < 0.05$). The changes in the peak systolic VEFR were less pronounced. Contrary to our expectations, the results of our study show that tonic pain induces an increase in the activity of NVC. This is in accord with the proposed modulatory influence of subcortical structures known to be activated during tonic pain on the mechanism of NVC. Our findings also imply that caution should be taken interpreting results of studies on pain relying on changes in regional cerebral blood flow as an indicator of neuronal activity such as blood-oxygenation-level dependent functional magnetic resonance imaging.

Keywords: cerebral circulation, evoked cerebral blood flow, tonic pain, cold pressor test, transcranial Doppler, neurovascular coupling, visual stimulus

Identification of microRNAs regulating expression of Arc protein in hippocampal neuron cultures

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Objectives of the study:

1. To identify microRNAs (miRNAs) that regulate Arc expression in hippocampal neurons.

2. To evaluate the effects of miRNAs on Arc protein expression.

Bioinformatics was used to predict miRNA binding sites within the Arc mRNA. Luciferase assays were used to identify potential functional miRNAs. Selected miRNAs which target specific sequences of the Arc 3'-UTR have the potential to reduce ARC protein expression.

Hippocampal neurons were obtained from Rattus norvegicus, and were cultured under optimal conditions for 7 days. The neurons were then transfected with individual selected DsRed tagged miRNAs, and with scramble miRNA and miRNAs which target non-specific sequences of the Arc 3'-UTR as controls. The neurons were then incubated for 3 days before examination of Arc and miRNA expressions. The neurons were treated with BDNF before Arc immuno-staining and F-actin phalloidin staining. Fluorescent microscopy was applied for imaging. Image processing was carried out. The net signal intensities of Arc protein and miRNAs were reported. Signal-to-Noise Ratios from Mean/SD of each cytoplasm and nucleus were calculated. Scatter Plots showing the distributed expression for each miRNA versus Arc protein were carried out. Statistical analysis was used to obtain sig-difference.

Statistical analysis of raw data indicates that transfection of the miRNAs reduced relative expression of endogenous Arc protein in the cytoplasm of hippocampal neurons exposed to BDNF as follows: miR326 > miR34a > miR19a > miR193a > controls.

MiR326 exhibited strongest regulation of Arc protein expression in hippocampal neuronal cultures, followed by miRs34a, 19a and 193a, respectively, when compared to controls.

Keywords: miRNAs, Arc protein, hippocampal neurons, BDNF (Brain-derived neurotrophic factor)

Impaired autophagy: a shared feature between neurodegenerative diseases and progressive myoclonus epilepsies

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Research into the molecular bases of neurodegenerative diseases has progressed, and therapies are being developed to combat the causes rather than only symptoms. On the basis of the observation that progressive myoclonus epilepsies (PMEs) and neurodegenerative diseases (NDs) share a common feature of neurodegeneration, we propose that the two pathologies might also share some common underlying molecular pathologies. It is well documented that autophagy is overloaded or impaired in neurodegenerative conditions, and it also is impaired in some of the PMEs, the clearest example is given by EPM2 (Lafora disease). We suggest that the common symptoms of PMEs and NDs might reflect a common cellular and molecular pathology. Specifically, we forsee and explain how impaired autophagy could produce a vicious circle, leading to oxidative stress, protein aggregation and finally neuronal death. Given the similarities between NDs and PMEs, we suggest that probing and targeting autophagy could be helpful for PME patients, at least as an adjunct therapy.

Reference:

Polajnar M and Žerovnik E (2011). Impaired auhophagy: in common to neurodegenerative diseases and progressive myoclonal epilepsies. Trends in Molecular Medicine, in print.

Keywords: neurodegeneration, impaired autophagy, protein aggregation, therapy, progressive myoclonus epilepsies PMEs

The effect of perinatal treatments with 5-hydroxytryptophan and tranylcypromine on central 5HT concentrations and metabolism in adult rats

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Serotonin (5HT) is a biologically active amine present in mammals in brain and peripheral tissues. Autism is a neurodevelopmental disorder in which 5HT homeostasis is disturbed both centrally and peripherally, but the relationship between the 5HT disturbances in the two compartments is not understood. In an attempt to explore the relationship between disturbed peripheral 5HT homeostasis and central 5HT functioning, we exposed a developing rat brain to increased 5HT concentrations, by the treatment of rats with subcutaneous injections of the immediate 5HT precursor 5-hydroxy-L-tryptophan (5HTP, 25 mg/kg), a non-selective MAO inhibitor tranylcypromine (TCP, 2 mg/kg), or saline, from gestational day 13 to post-natal day 21. 5HTP significantly raised only peripheral 5HT concentrations, while TCP induced significant 5HT elevations in both compartments. The effects of the mentioned treatments on central 5HT levels and metabolism were then studied in adult rats. Cortical and midbrain 5HT, tryptophan and 5-hydroxyindol acetic acid concentrations were determined by HPLC in twelve 5HTP-, eight TCP-, and ten saline-treated rats. In 5HTP treated animals, modest decrease in 5HT concentration without effects on 5HT metabolism was observed in cortex, presumably due to hyperserotonemia-induced loss of 5HT terminals during brain development. In TCP treated rats, altered 5HT metabolism with strong decrease in 5HT concentrations was observed both in cortex and midbrain. We suppose that the inhibition of one 5HT-regulating element induced compensatory changes in the expression/activity of other 5HT-regulating elements leading to a permanently disturbed 5HT homeostasis in rats treated with MAO inhibitor.

Keywords: serotonin, 5HT metabolism, HPLC, brain, 5-hydroxytryptamine, tranylcypromine, rats

Changes at GABA-A receptors induced by long-term zolpidem treatment in primary culture of rat cerebellar granulle neurons

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Gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the mammalian brain, fulfills most of its physiological actions via GABA-A receptors. GABA-A receptors possess binding sites for a variety of different drugs, including clinically relevant benzodiazepines, barbiturates, general anesthetics and neurosteroides. Occupancy of these receptors by different drugs leads to regulatory changes often affecting receptor expression and/or function.

The aim of this study was to further explore the mechanisms leading to adaptive changes in GABA-A receptors following their prolonged exposure to zolpidem, a positive allosteric modulator of GABA-A receptors. Imidazopyridine zolpidem is the most widely prescribed non-benzodiazepine hypnotic, with preferential, although not exclusive, binding for receptors containing alpha1 subunit. It was suggested that drugs with high selectivity for alpha1 containing receptors produce, upon repeated treatment, less tolerance and dependence than classical benzodiazepines.

As an extension of our previous work, we treated cerebellar neuronal cells isolated from 8-days old rats with 10 microM zolpidem during 48 h. Results demonstrate that prolonged treatment of these cells with zolpidem induced changes neither in GABA-A receptor number nor in expression of alpha1 subunit mRNA. On the other hand, long-term exposure of these cells to zolpidem produced the functional uncoupling between GABA and benzodiazepine binding sites on GABA-A receptor complex as evidenced by a decreased ability of GABA to stimulate [3H]flunitrazepam binding.

We can assume that chronic zolpidem treatment might also induce tolerance if this mechanism is responsible for the development of tolerance following chronic administration of classic benzodiazepines.

Keywords: GABA-A receptors, cerebellar granulle neurons, zolpidem

Brain and spinal cord affected by amyotrophic lateral sclerosis induce different growth factors expression patterns in neural and mesenchymal rat stem cells

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons. The modern therapeutic approach includes transplantation of stem cells with the aim to slow down disease progression. This study reports that central nervous system affected by ALS induce production of growth factors in stem cells. Upon stimulation with ALS brain and spinal cord protein extracts, mesenchymal stem cells (MSC), neural stem cells (NSC) and fibroblasts exhibited different expression patterns of growth factors. Nerve growth factor and brain-derived neurotrophic factor were upregulated in both NSC and MSC cultures with ALS specific pattern. Fibroblast growth factor 2, insulin-like growth factor and glial-derived neurotrophic factor were upregulated in NSCs in an ALS-dependent manner, while the same factors were ALS-independently downregulated in MSCs. Vascular endothelial factor upregulation was restricted to MSCs and fibroblasts. Surprisingly, ALS spinal cord, but not the brain extract, upregulated brain-derived neurotrophic factor in MSC and glial-derived neurotrophic factor in NSC cultures. These results suggest that inherent characteristics of different stem cell populations define their healing potential and raise the concept which implicates importance of ALS environment in stem cell transplantation.

Keywords: amyotrophic lateral sclerosis, stem cells, neurotrophic factors, regenerative neuroscience

Alzheimer-related neuronal changes and molecular chaperones

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The most conspicuous feature of many neurodegenerative disorders including Alzheimer's disease is the occurrence of protein aggregates in ordered fibrillar structures known as amyloid found inside and outside of brain cells. Amyloid formation is thought to arise from partial unfolding and exposure of hydrophobic surfaces that are normally buried in the core of a folded protein, thus increasing attractive forces among protein molecules. Mutations that increase the production of amyloid cause rare, early-onset forms of AD. Chaperones are one of the best examples of multifunctional proteins and their production against neurodegeneration may result from one or more of their activities in cells, perhaps in addition to their ability to inhibit fibril formation directly. Expression pattern analysis indicates that postmortem cortical tissues from Alzheimer's disease cases contain significantly higher levels of the Hsp90/70 and this elevation is associated with the disease pathology. In addition, the monoclonal antibody AT8 identifies distinct forms of tau in Alzheimer's disease. Monoclonal antibody TG3 stains neuritic plaques and neurofibrillary tangles but does not react with tau from normal human biopsy tissue; therefore exhibiting its high degree of specificity for AD pathology. TG3 also visualizes neurons susceptible to tangle-formation and thus seems to be a marker of early AD pathology.

Protein misfolding is believed to be the primary cause of Alzheimer's disease. Clearly, the effects of chaperones in multiple cellular pathways will have to be deciphered in order to understand which of these effects are primary and which are secondary in protection against neurodegeneration.

Keywords: Alzehimer disease, molecular chaperones, immunohistochemistry, Western blot

STAM2 expression in the central nervous system during embryodevelopment

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Signal transduction and receptor downregulation by endocytic pathway are crucial processes in the mammalian embryonic development and normal functioning of nervous system. STAM2 is a tyrosine-phosphorylated protein suggested to be involved in cargo selection during endocytic pathway, regulation of exocytosis and intracellular signaling. The aim of the research was to determine STAM2 expression pattern in the mouse embryos and the brain of newborn mice in order to get insight into its possible role in the embryodevelopment and neurodevelopment. Gene trap method was used to create a mutant mouse line with integration of promotorless βgeo (lacZ-neomycin phosphotransferase fusion) gene in the second intron of STAM2 gene, enabling analysis of its expression by histochemical staining for ß-galactosidase. STAM2 expression was present in the mouse embryos after developmental stage E9.5 in the primitive hindgut, notochord, future central nervous system and heart, and toward the end of gestation in some organs of the endocrine system and in the covering epithelia. In the nervous system it was located in the floor, roof and basal plates of the developing neural tube, and in the developing cortex of the telencephalon, hippocampus and olfactory bulbs. STAM2 was strongly expressed in the forebrain cortex, hippocampus and olfactory bulbs of newborn mice. The results of this research showed a possible role of STAM2 in the development of the nervous system, heart, endocrine glands and epithelial tissues that could be related to its role in signal transduction regulation by endocytic pathway.

Keywords: STAM2, mouse, development, central nervous system, gene trap

The cross-road between mechanisms of protein folding and aggregation by studies of stefin B (Y31) wild-type variant and its H75 mutant

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For the case of the recombinant human stefin B (Y31) wild-type variant and its point mutation H75W(Y31) the role of the aromatic residue at site 75, protein stability and the mechanism of folding on the amiloid-fibril formation reaction were investigated. Mutant H75W which has of lower conformational stability in comparison to the wild-type (wt) human stefin B (E31) showed somewhat lower propensity to fibrillise. Revealing protein conformation at the cross-road between folding and aggregation, human stefin B wild-type iso-form (Y31) was found to fold at pH 4.8 in three kinetic phases. The pH conditions which lead to the formation of the native state intermediate are similar to those for amyloid fibril formation of human stefin B wild-type iso-form, meaning that the process of folding and amyloid-fibril formation share the same structural intermediate. At pH 5.8 and 7.0 protein folded to the native state in four kinetic phases over two intermediates. Stefin B mutant H75W(Y31), however, folded only to intermediate states at all pH values: 4.8, 5.8 and 7.0. At pH 4.8 and 5.8, H75W(Y31) folded in one kinetic phase to the intermediate or the "molten globule" type, which leads to the conclusion that its mechanism of folding differs to that of folding of human stefin B (Y31) wild-type variant at the same pH. At pH 7.0 the mutant H75W(Y31) folded in three kinetic phases to a structure of the intermediate 2, analogous to folding of the human stefin B (Y31) wild-type variant at pH 4.8.

Keywords: amyloid fibril formation, mechanism of folding, intermediate "molten globule", kinetic phase

Involvement of key components of Wnt signaling in human astrocytic brain tumors

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Molecular landscape of human brain tumors is still inadequately explained. In the present study key players of wnt signaling, adenomatous polyposis coli (APC), axin (AXIN1) E-cadherin (CDH1) and beta-catenin (CTNNB1) were investigated in the set of human astrocytic brain tumors. Genetic changes were tested by PCR/loss of heterozygosity (LOH). Beta-catenin (CTNNB1) was investigated by heteroduplex method. Relevant proteins were investigated by immunohistochemistry.

Changes of APC gene were frequent and distributed among different tumor grades, with glioblastomas showing the highest percentage (60%). LOHs were also detected in 20% of diffuse astocytomas (II). AXIN1 was tested with D16S521 microsatellite marker and LOH was found in 10% of glioblastomas (IV) while astrocytomas of grades I, II and III did not demonstrate LOH. The investigation on beta-catenin demonstrated 8.3% of samples with potential mutations. Changes of CDH1 gene were not detected in our study. In 31% of glioblastomas and 22% of astrocytomas downregulation of axin proteins was detected. The majority of glioblastomas (69%) had axin localized in the cytoplasm, while in 31% it was observed in the nucleus. Astrocytomas also demonstrated nuclear localization in 44% of cases. In 50% of glioblastomas and 56% of astrocytomas upregulation of beta-catenin was noted. Our findings on changes of the wnt molecular components contribute to better understanding of human astrocytic brain tumors' genetic profile.

Keywords: astrocytic brain tumors, wnt signaling, APC, AXIN1, beta-catenin, CDH1

Complex structural composition of accumulated GD1a species in brain tissue of GD3 synthase knockout mice

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Gangliosides, glycosphingolipids containing sialic acids, are found with great diversity and variety in mammalian central nervous system. Many of the physiological functions of gangliosides have been elucidated using knock-out (KO) mouse models.

The aim of the study was to systematically characterize the structures of brain gangliosides in St8sia1 KO mice in comparison to wild-type (wt) mice. The St8sia1 mice lack GD3 synthase with consequent deficiency in b-series gangliosides. In this study, frontocerebral tissue of 3 St8sia1 and 3 wt mice was homogenized and used for total ganglioside extraction. Separation of ganglioside mixture was performed using high-performance thin-layer chromatography (HPTLC), also utilized for preparative isolation of separated fractions for subsequent mass spectrometry (MS) analysis. MS screening and sequencing analyses (MS1; MS/MS) were performed on a High Capacity Ion Trap Ultra mass spectrometer coupled with a NanoMate robot incorporating ESI 400 Chip technology. All mass spectra were acquired in the m/z range 100-3000. Biochemical phenotype of St8sia1 mice was confirmed by finding complete absence of GD3, GD2, GD1b, GT1b and accumulation of GD1a and GM1. In accumulated GD1a HPTLC migrating fraction from St8sia1 mice brain, a higher number and proportion of individual molecular species comparing to the wt was determined by MS. Detected GD1a species differed by a composition of the ceramide moiety and a higher abundance of acetylated and fucosylated monosialo/disialo-ganglioside structures was revealed. The presence of described ganglioside structures in brain tissue of St8sia1 mice indicates structural modifications i.e. possible alternative synthetic pathways as a consequence of GD3 synthase deficiency.

Keywords: brain gangliosides, mass spectrometry, GD3 synthase deficiency

Na,K-ATPase beta3 subunit gene expression is altered in brain tissue of ganglioside deficient mice

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Na,K-ATPase, or sodium pump, maintains steep electrochemical gradients across cell membranes. This pump is an oligomer consisting of alpha and beta subunits; alpha being the catalytic subunit, while beta is required for biogenesis and correct folding of the alpha subunit in the membrane. Beta subunit has also been shown to have a role in cell-cell adhesion, however all potential partners interacting with this subunit have not yet been identified.

It has been previously demonstrated that certain gangliosides, sialic acid-bearing membrane glycosphingolipids especially abundant in brain tissue, activate Na,K-ATPase. The aim of this study was to examine whether changed ganglioside composition of the cell has an effect on Na,K-ATPase beta3 subunit (Atp1b3) gene expression. We used cerebellar and hippocampal tissue of two ganglioside deficient knockout mouse models, St8sia1 and B4galnt1, whose phenotypes include impaired peripheral nerve regeneration, dysmyelination, axonal degeneration, impaired motor coordination and hyperactivity. We performed quantitative real-time PCR and compared relative gene expression levels for Atp1b3 in both tissues for the two mouse models in comparison to wild-type mice. We found that in St8sia1 model gene expression of Atp1b3 was downregulated for cerebellum and hippocampus. Interestingly, in B4galnt1 mice the expression for the same gene was upregulated in both tissues.

We suggest that specific ganglioside membrane composition probably affects Na,K-ATPase beta subunit expression which could lead to changes in assembly of the whole pump resulting in decrease or increase of its catalytic activity. This assumption needs confirmation by measuring Na,K-ATPase enzymatic activity in brain tissue of ganglioside deficient mice.

Keywords: Na,K-ATPase, brain gangliosides, ganglioside deficient mice

Decreased adult brain neurogenesis in the rat overexpressing ICER II (TG ICER II)

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ICER (Inducible cAMP Early Repressor) proteins are strong, endogenous antagonists of CREB (cAMP Responsive Element Binding). It has recently been demonstrated that CREB inhibition negatively affects the maturation and survival of new neurons in the adult mouse hippocampus [1]. Herein, we decided to test whether overexpression of ICER II in the transgenic rat may have an impact on the adult brain neurogenesis.The rats were created by conventional transgenesis with ICER II overexpression driven selectively in neurons by the Synapsin I gene promoter. The neurogenesis in both major brain areas, namely dentate gyrus of the hippocampus and subventricular zone-olfactory bulb pathway were analyzed by immunofluorescence approach following i.p. injection of bromodeoxyuridine (BrdU) a DNA precursor. Neuronal markers, i.e., doublecortin (DCX) that labels immature neurons and NeuN to label the mature ones were also employed. We have observed a decrease in the number of double stained BrdU+/DCX+ neurons in the hippocampus of the 3 month TG ICER II, as compared with the wild type (WT) littermates. Notably, the total number of BrdU+ cells was not altered. Furthermore, in the olfactory bulb a lower number of BrdU+/NeuN+ cells was demonstrated in 6 month old TG ICER II rats in comparison to the WT. These results confirm important role of CREB/ICER system in the maturation of the newly born neurons in the adult brain.

Reference:

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Keywords: ICER, adult brain neurogenesis

Different intracellular localization of STAM adaptor proteins in neurons

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STAM1 and STAM2 (Signal transducing adaptor molecule) are phosphotyrosine proteins, members of the endosome associated complex ESCRT-0, involved in sorting of mono-ubiquitinated endosomal cargo for degradation in the lysosome. Apart the suggested role in enodosomal trafficking, STAM possible roles are in cytokine signaling as well. STAM are phosphorylated on tyrosine upon stimulation with a variety of cytokines and growth factors. They are associated with JAK2 and JAK3 tyrosine kinases, and are involved in the regulation of intracellular signal transduction for DNA synthesis and c-myc induction mediated by IL-2 and GM-CSF.

In order to clarify which cell types in mice brain highly expressed STAM proteins we found colocalization of STAM proteins with neuronal markers MAP2 and NeuN, but very weak colocalization with GFAP, O4 and Iba1 as astrocyte, oligodendrocyte and microglia cell marker. STAM1 and STAM2 proteins are scattered through the soma and nerve fibers, showing punctuate appearance and high fluorescence intensity. Together with previous observations that ESCRT-0 proteins are tightly associated on the early endosome, we are indicating that punctuate structures in the cytoplasm are the early endosomes. Surprisingly, we also found storng and homogenous STAM2 signal in the neuron nuclei using immunohistochemical staining method with three different commercial antibodies.

As several proteins involved in ESCRT complex play additional role in the cell nucleus, these results suggest that some proteins may play a dual role in sorting proteins for lysosomal degradation and in regulating nuclear gene expression. One of these proteins with possible dual role might be STAM2.

Keywords: STAM2, STAM1, ESCRT, endosome, nucleus, protein localization

Inflammatory and neuroprotective proteins in the thalamus following traumatic brain injury in the rat

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Thalamus is the brain region whose damage has been well documented after both human and experimental traumatic brain injury (TBI). Molecular mechanisms which lead to post-traumatic cell death or support the neuronal survival in the thalamus, in this entity, have not yet been fully characterized. Therefore, aim of this study was to determine the changes in several markers of inflammation and also cell protection following experimental TBI in the rat. Experiments were carried out on adult male rats. TBI of moderate severity was performed over the left parietal cortex using the lateral fluid percussion brain injury model. Sham-operated animals were used as the control group. Rats were sacrificed 24 h after the TBI induction and the thalami were processed for the Western blot analyses of different protein expressions. TBI caused significant increases of the cyclooxygenase-2, inducible nitric oxide synthase and heat shock protein 70 expressions in the thalamus of rats with TBI in relation to the values registered in sham-operated animals. Thalamic expressions of inhibitory kappa B alpha and brain derived neurotrophic factor were unaffected by TBI in our experimental conditions.

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Keywords: traumatic brain injury, thalamus, inflammation, neuroprotection, rat

The effect of propofol on BDNF and TrkB expression in postnatal rat brain: neuroprotection via Akt/ERK signaling

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The use of general anesthetics deserve careful consideration because they can induce neurodegeneration in developing brain. It is known that neurotrophins, in addition to their role in neuronal survival can mediate neurotoxic effects of anesthetics.

We analyzed whether propofol when given to postnatal Wistar rats (P14) modifies survival and synaptic plasticity. We examined BDNF, its receptor TrkB and downstream kinsases Akt and ERK1/2. Western blot analyses was used to evaluate whether the effects of propofol (25 mg/kg i.p.) on these targets differ in cortex and thalamus during first 24 hours (0, 1, 2, 4, 8, 16, 24 h). Fluoro-Jade B staining was used for detection of degenerating neurons.

We demonstrate that propofol regulates intracellular pathways linked to survival and neuroplasticity in an area-specific manner. In cortex, propofol treatment decreased the levels of mature BDNF, pTrkB and pAkt, but increased the levels of pERK1/2. Propofol had opposite effect in thalamus: increased the levels of BDNF, pTrkB and pAkt while the levels of pERK1/2 were decreased. Propofol did not change levels of total Akt and total ERK1/2. Fluoro-Jade B staining showed no degenerating cells in any examined structure.

Our results suggest that in addition to pAkt, whose role in neuronal survival has been well characterized, pERK1/2 has important pro-survival role as well. In our model interplay between pAkt and pERK1/2 seems to be region specific. These new insights about differential pro-survival activation pattern of Akt and ERK1/2 in distinct brain regions could help our understanding of anesthetic actions in vivo.

Keywords: neurotrophins, rat brain, postnatal development, neuroprotection

Long-term exposure of recombinant GABAA receptors to neurosteroid dehydroepiandrosterone sulfate (DHEAS)

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Long-term exposure to a variety of drugs including benzodiazepines, barbiturates and steroids induces different adaptive changes in GABA-A receptors, the major fast inhibitory neurotransmitter receptors in the mammalian brain. In order to better understand the underlying mechanisms, human embryonic kidney (HEK) 293 cells stably expressing recombinant alphalbeta2gamma2S GABA-A receptors (the most common type of GABAA receptors) were exposed for 24 and 48h to neurosteroid dehydroepiandrosterone sulfate (DHEAS). The aliquots of membrane preparations obtained from control and DHEAS-treated cells were used in the saturation binding studies with [3H]flunitrazepam and [3H]t-butylbicyclоorthobenzoate ([3H]TBOB) to determine the possible changes in the binding parameters (Kd and Bmax), as well as in the functional interactions between GABAA receptor binding sites. Exposure of HEK 293 cells stably transfected with recombinant alphalbeta2gamma2S GABA-A receptors to 100 microM DHEAS for 24 and 48h have not changed the maximum number nor the affinity of the binding sites for benzodiazepines and convulsants. Moreover, no significant differences between the groups were observed in the potency (IC50) of DHEAS to modulate [3H]TBOB binding or in the efficacy (Emax) of GABA to potentiate [3H]flunitrazepam binding. These results have demonstrated that unlike benzodiazepines, chronic DHEAS treatment does not affect expression and functional coupling of GABA-A receptor binding sites. If, as previously suggested, allosteric uncoupling is related to the development of functional and behavioral tolerance following prolonged treatment with GABA-A receptor ligands (as in the case of benzodiazepines), then one might presume that chronic treatment with DHEAS will not produce tolerance.

Keywords: GABA-A receptor, DHEAS, long-term treatment, expression, allosteric uncoupling

Sex differences in the brain gene expression in WT and SF-1 knockout mice determined by microarray analysis

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Male and female brains differ as a consequence of gonadal hormones, but also to some extent processes that are hormone independent. Steroidogenic factor 1 knockout (SF-1 KO) mice are born without gonads and adrenal glands, and die shortly after birth due to adrenal insufficiency. They can be rescued with early corticosteroid injections followed by adrenal transplantation. Since SF-1 KO mice are not exposed to endogenous sex steroid hormones, they provide an important model for studying hormone independent sex differences in the brain.

To begin to examine gene expression in this hormone-independent model, Affymetrix GeneChips® were used to examine a large region of adult mouse brain, containing the preoptic area, hypothalamus, amygdala, hippocampus and part of the cortex from WT control and SF-1 KO mice. Statistical analyses revealed six differentially expressed genes: four of them were Y chromosome linked and were expressed at higher levels in both male genotypes. The other two were X chromosome linked; both of them were expressed higher in females than in males of both genotypes. Microarray results were validated by quantitative RT-PCR. These results confirmed that Y-linked genes were expressed only in males and both X-linked genes were greater in females. Interestingly, the X-linked gene Xist that was not expressed in brains from WT males was expressed in male SF-1 KO brains, suggesting that sex steroid hormones during development might influence the regulation of the Xist gene in brain.

Keywords: Steroidogenic factor 1, mouse, brain, sex differences, microarray

Glucose-oxygene deprivation induces qualitative and quantitative changes in histamine uptake into cultured rat astrocytes

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Apart from its classical role in allergy and inflammation, histamine is also an important neurotransmitter in central nervous system where it is involved in regulation of several key neurophysiological functions such as the sleep-wake cycle. After its release into the synaptic cleft, histamine is taken up by the presynaptic neurons and surrounding glial cells to be either metabolized or recycled. Despite the importance of histamine transport in termination of its action, exact molecular mechanisms and their regulation remain largely unresolved. As an electrically charged molecule, histamine is most probably taken-up by a carrier-operated transport system, but selective histamine transporter remains elusive.

We established that at least two types of histamine transport exist in cultured rat astrocytes. Apart from the active component, which has characteristics of Na+-dependent, Cl—independent and ouabain-sensitive bidirectional transport, with Michaelis-Menten constant (Km) of 3.5 microM and a maximal rate (Vmax) of 7.9 pmol/mg protein/min, there is also an ouabain-insensitive, temperature- and Na+-independent passive component (electrodiffusion) with some similarities to the so called uptake 2. When astrocytes were exposed to glucose-oxygen deprivation (OGD), an in vitro model of ischaemic stroke, they retained their capability to take up histamine but they had a reduced rate of histamine transport and ouabain-sensitivity was lost. There was also an increase in the amount of released histamine. Although histamine transport remained Na+ –dependent, there was a clear shift from the two-component process (active transport and electrodiffusion) to one-component process (electrodiffusion only).

Keywords: uptake, histamine, glucose-oxygene deprivation, astrocytes, rat

Promoter DNA methylation before the onset of neurogenesis is dependent on cluster structure, and regulates allocation of isoforms gene expression in each Protocadherin cluster

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Clustered Protocadherins (Pcdhs) are organized into three gene clusters, Pcdha, Pcdhb and Pcdhg, and encode nearly 60 isoforms of diverse transmembrane proteins that are predominantly expressed in the nervous system. Interestingly, the majority of the Pcdh isoforms are expressed combinatorially in individual neurons. The unique patterns of their expression could generate neuronal diversity, and implicate the potential that the Pcdh isoforms contribute to specify different neuronal identities and connectivity. DNA methylation is the major epigenetic modification, and is involved in the establishment of cellular identities. It has been revealed that the promoter DNA methylation of Pcdha genes is correlated with its expression in vitro, however, their relationship in vivo is unclear. Previous study showed that genetic conversion of Pcdha cluster disturbed the differential expression frequency of Pcdha isoforms in each neuron. The Pcdh genes have their own promoters, here we found that the levels of their promoter DNA methylation was correlated with the change of expression frequency. In addition, we revealed that the promoters of Pcdh isoforms were methylated by de novo DNA methyltransferase Dnmt3b from E3.5 to E9.5, dependent on its cluster structure. Furthermore, we found that the expression level of each Pcdh isoform was changed in Dnmt3b-deficient brains. These results suggested that epigenetic modification before the onset of neurogenesis could regulate the appropriate allocation of the distinct Pcdh genes expression.

Keywords: clustered Protocadherin, neuronal diversity, epigenetics

Histamine H3 receptor in astrocytes: role in NT-3 synthesis

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Histamine (HA) is an important neurotransmitter and neuromodulator in the brain. Astrocytes express histamine H1 and H2 receptors thus indicating the existence of a functional role of HA in these cells. Astrocytes produce neurotrophin-3 (NT-3), which is susceptible to regulation by the monoamine neurotransmitters (Mele et al., 2010). So far, the impact of HA on the regulation of astrocytic NT-3 synthesis has not been studied in detail.

HA potently and transiently elevates NT-3 expression and protein levels in cultured rat cortical astrocytes. As shown by real-time PCR and binding studies, in addition to H1 and H2 receptors astrocytes express also H3 receptor. The identified receptor is coupled to Gi/o protein to inhibit adenylyl cyclase, modulate PLC/PKC (but not CAMK II) signaling and MAP kinase activity. Using pharmacological tools, selective for histamine receptors and intracelullar systems, it was demonstrated that not only H1 and H2 receptors but also H3 receptor significantly participates in the stimulatory effect of HA on NT-3.

In conclusion, the synthesis of astrocytic NT-3 induced by HA is a receptor-mediated process, which is fine-tuned via subtle modulation of parallel histaminergic pathways: H1/PLC/PKC/CaMKII, H2/cAMP/PKA and the novel H3/Gi/o-protein/PKC pathway. H1, H2 and H3 receptor cascades converge at the level of MAP kinase activity (Jurič et al., 2011, in press).

Keywords: histamine, histamine H3 receptor, neurotrophin-3, astrocytes

Brain perfusion changes in Parkinson's disease: the effect of dopaminergic treatment

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The aim of the study was to investigate changes in brain activity in early Parkinson's disease (PD) patients using brain perfusion Single Photon Emission Tomography (SPECT) before and after dopaminergic treatment.

Brain perfusion imaging with 99mTc-ECD SPECT was performed in twelve patients with early, »de novo« PD (age: 55 ± 9 yrs; male/female: 8/4; disease duration: 22 ± 8 months) prior to (PD1) and following the 3-months treatment with dopamine agonist pramipexole (PD2), and in 16 normal controls (NC) (age: 55 ± 7 yrs; male/female: 10/6). Voxel by voxel image analysis with statistical parametric mapping (SPM8) was applied to compare relative perfusion in PD1 vs. NC, PD1 vs. PD2 and PD2 vs. NC. The SPM(t) values were thresholded at the $p < 0.01$ uncorrected level and clusters with minimum size 100 voxels were considered significant in all reported results.

We found hypoperfusion in PD1 vs. NC predominantly in parietal brain regions and hyperperfusion in brain stem, cerebellum and in the right medial temporal cortex spreading towards basal ganglia. In treated PD patients (PD2 vs. NC) hyperperfusion was found in the same regions as in PD1, with much less hypoperfusion in parietal cortex. By comparing PD1 vs. PD2 it was found that pramipexole causes an increase in parietal perfusion. In no brain region pramipexole caused decrease of brain perfusion.

PD is associated with hypoactivity in parietal cortex and hyperactivity in brain stem, cerebellum, medial temporal cortex and basal ganglia. Dopaminergic treatment diminishes parietal hypoperfusion but has no effect on hyperactive brain regions.

Keywords: SPECT, Parkinson's disease, dopaminergic treatment

Neuroprotective effect of quercetin against hydrogen peroxide-induced cell death in the culture of P19 neurons

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Oxidative damage by reactive oxygen species (ROS), secondary intermediates in intracellular signaling, has been implicated in dysfunctions of mammalian brain in numerous diseases and injuries. The aim of this study was to better understand the molecular mechanisms of neurodegeneration induced via oxidative stress and the protective effect of flavonoid quercetin on the neuronal cell death induced by hydrogen peroxide (H2O2) exposure for 24 hours. The effect of quercetin on H2O2-induced injury was investigated in the culture of P19 neurons, differentiated from the P19 mouse embryonal carcinoma cells in the presence of retinoic acid. Complete neuronal maturation of P19 neurons was confirmed by immunofluorescence staining against neuron specific marker beta-III tubulin. As determined by methylthiazolyltetrazolium bromide (MTT) assay, treatment with H2O2 (1.5 mM) significantly decreased the cell viability. In the dose-dependent manner, quercetin reversed the toxic effect of H2O2. Release of lactate dehydrogenase (LDH) from H2O2-damaged cell membranes was also significantly reduced in the presence of quercetin indicating that the P19 neurons were less vulnerable in the presence of quercetin. While H2O2 treatment markedly induced the production of ROS, increase in cell survival in the presence of quercetin was accompanied by a significant decrease in ROS production. Quercetin also decreased the activities of key apoptotic markers caspase-3/7. The obtained results suggest that quercetin, probably due to the interfering with the apoptotic pathway, can act as survival factor in neuronal cells. In light of these findings, beneficial effects of quercetin could be taken into account for potential therapeutic uses in neuroprotection.

Keywords: quercetin, oxidative stress, neuroprotection, P19 neurons

Experimental ischemic stroke: changes in lipid peroxidation and antioxidant enzyme activities in rat cortex

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In the present study, we examined the effect of middle cerebral artery occlusion (MCAO) on lipid peroxidation and antioxidant enzymes (superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)) activities in rat cortex.

Right MCAO was induced in male Hannover Wistar rats (250-350 g) for 1 hr. After 23 hrs of reperfusion, ischemic animals were sacrificed and the levels of lipid peroxidation, SOD and GSH-Px activities were determined spectrophotometrically in the right and left cortex. Sham operated animals served as the control group.

Focal cerebral ischemia significantly increased the level of thiobarbituric acid-reactive substances (TBARS) in the right cortex in comparison to the control group while SOD and GSH-Px activities were not affected. MCAO did not cause contralateral cortical damage since TBARS levels as well as antioxidant enzymes activities were not statistically different in the left cortex.

Our results showed that transient focal cerebral ischemia caused oxidative lipid damage in the right cortex of rats after 23 hrs of reperfusion without affecting antioxidant enzyme activities. Changes in activities of above mentioned parameters in left cortex did not reach the level of significance.

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Keywords: focal cerebral ischemia, lipid peroxidation, SOD, GSH-Px, cortex, rat

LPS-induced IL-6 secretion enhance proliferation of human myoblasts

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Cultured human myoblasts robustly secrete IL-6 in basal and stimulated conditions. It has been demonstrated that IL-6 stimulates myoblast proliferation, and therefore the physiological meaning of this secretion might be autocrine and paracrine stimulation of the muscle regeneration process. However, we found that under our experimental conditions, addition of commercially available synthetic IL-6 had no significant effect on the in vitro proliferation neither in human myoblasts nor in the C2C12 muscle cell line. On the other hand, myoblasts treated with the conditioned medium collected after stimulation of the IL-6 secretion from myoblasts by the endotoxin lipopolisaccharide (LPS), have shown increased proliferation rate as assayed by BrdU proliferation assay. Our finding indicates that IL-6-mediated stimulation of myoblasts proliferation is complex and depends on some additional factors secreted from the stimulated myoblasts beside IL-6. It is possible that LPS induces secretion of soluble form of IL-6 receptor subunit -sIL-6Ra- in the medium, which in turn activates IL-6-mediated stimulation of cell proliferation by the principle of transsignaling.

Keywords: myoblast, proliferation, bromodioxiuridine (BrdU), endotoxin lipopolisaccharide (LPS), interleukin-6 (IL-6)

Interspecies differences in PSA-NCAM zones in adult fish brain

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The neural cell adhesion molecule (NCAM) plays important role in adult neurogenesis, survival and migration of neuronal steam cells. Reversible attachment of polysialc acid (PSA) to extra cellular domain of NCAM promotes migration which is limited to rostral olfactory stream in the adult brain of mammals. Non-mammalian vertebrates poses much higher potential for regeneration due to constitutive adult neurogenesis, apoptotic elimination of damaged cells and efficient migration, differentiation and integration of newly derived cells in existing neural network.

We compared expression of PSA-NCAM in sturgeon (Acipenser ruthenus), carp (Cyprinus carpio), trout (Salmo gairdneri), catfish (Silurus glanis), perch (Perca fluviatilis) and pike (Esox lucius) brains with two different antibodies (mAb735 and clone 2-2B). PSA-NCAM zones in carp, trout and perch brain extend from telencephalon into cerebellum and mesencephalon. From the level of rostral mesencephalon toward spinal cord extensive PSA-NCAM zone exist in sturgeon and pike brain. While two antibodies give similar result on trout and pike brains, reactivity of mAb735 is limited to cerebellar proliferation zone in perch and carp. Also, carp and catfish brain, contrary to others, have proliferative zones in facial and vagal lobes - enlargement of nucleus solitarius connected with taste system and specialized palatal organ.

PSA-NCAM is just one of the markers that are indicating potential for regeneration, but its expression in rhombencephalon and spinal cord of sturgeon and pike could be promising for spinal cord injury studies.

Keywords: PSA-NCAM, migration, regeneration, fish brain

Complex gangliosides in fish brain

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Glycolipids are dominant glycans in vertebrate brains. Complex gangliosides GD1a, GD1b and GT1b are ubiquitously expressed on neurons while GM1 is marker of fibers in all reptilian, bird and mammalian brains. Complete lack of complex gangliosides does not interrupt brain morphogenesis but does affect long term myelin and axon maintenance due to interaction of GD1a and GT1b with oligodendrocyte ligand MAG. The same axon/myelin preserving interaction turns into regeneration obstructing in the case of injury in adult brain. In contrast to higher vertebrates, fish and amphibian brains are deficient in complex gangliosides but well known as regeneration-competent.

Using antibodies for GM1, GD1a, GD1b and GT1b we investigated difference in distribution of gangliosides in brains of sturgeon (Acipenser ruthenus), catfish (Silurus glanis), carp (Cyprinus carpio), trout (Salmo gairdneri), pike (Esox lucius) and perch (Perca fluviatilis). Contrary to other vertebrates that have uniform pattern of ganglioside expression, investigated species of Teleostei varied in ganglioside expression. All but one species (sturgeon) expressed GM1 as neuronal marker, not being present on fibers like in mammalian brain. Catfish, carp and trout express GM1 and GT1b on individual neurons in telencephalon, hypothalamic nuclea, granular neurons in cerebellum and stratum periventriculare in optic tectum. GD1a is almost undetectable in trout, GD1b in catfish and carp while GT1b in perch brain.

Brain of sturgeon has expression of complex gangliosides resembling one observed in higher vertebrates and its regeneration-competence could be farther investigated.

Keywords: gangliosides, fish brain, regeneration

The lack of association of GABRA2 polymorphism and alcohol dependence in Croatian population

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Gamma-aminobutyric acid-A (GABA-A) receptors, the major fast inhibitory neurotransmitter receptors in the brain, are implicated in the acute and chronic effects of alcohol including tolerance, dependence and withdrawal. Recent studies have suggested a linkage of alcohol dependence to the gene encoding GABA-A receptor alpha2 subunit (GABRA2), on chromosome 4p. Therefore we examined the association of alcohol dependence to rs9291283 (hCV8262290) polymorphism, located in the intron 3 of GABRA2 gene, that has been previously reported to be implicated in alcoholism. This study enrolled 379 (303 male and 76 female) alcohol-dependent (diagnosis of alcoholism was made according to DSM-IV criteria) and 226 (198 male and 28 female) healthy subjects of the Croatian origin. Alcohol-dependent patients were further subdivided according to the early/late onset of alcohol drinking, as well as to the presence/absence of aggressive behavior. Genotyping was performed by using TaqMan Real-Time allelic discrimination technique after extraction of DNA from the whole blood with a salting out procedure. There were no significant differences in the distribution of genotypes or in the frequency of different allele carriers for tested polymorphism between alcohol-dependent and control individuals, as well as between different subsets of alcoholics. The results have not provided supporting evidence for the potential involvement of the rs9291283 (hCV8262290) GABRA2 polymorphism in the alcohol dependence. However, since these are preliminary results, further studies with enlarged groups should be conducted in order to find a possible association of investigated polymorphism and alcoholism in Croatian population.

Keywords: alcoholism, GABA-A receptor, GABRA2 gene, polymorphism, Croatian population

TDP-43 regulates nuclear transport and RNA-binding proteins

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Transactive response DNA-binding protein 43 (TDP-43) is a predominantly nuclear, ubiquitously expressed RNA and DNA-binding protein. It has two RNA-binding domains, a Gly-rich domain important for protein-protein interactions, a nuclear localisation signal and nuclear export signal sequence. It recognises and binds to UG repeats and is involved in pre-mRNA splicing, mRNA stability and metabolism of microRNA. It is essential in early embryonic development and also involved in neurodegeneration processes in some neurological disorders. TDP-43 proteinopathies are known to occur in subtypes of ALS, FTLD, Alzheimer's disease and some other neurodegenerative disorders. It is yet not known whether TDP-43 aggregates are toxic or form in order to protect the cells and if they represent a loss of normal TDP-43 function or a gain of some new function. Several TDP-43 mutations which particularly occur in Gly-rich region are found in familial and sporadic ALS and FTLD cases.

We have done a proteomic study in which we compared protein levels in intact HeLa cells and HeLa with siRNA mediated TDP-43 knockdown by mass spectrometry and western blot analysis. Our results indicate a major role of TDP-43 in RNA metabolism since TDP-43 knockdown shows to have an impact, whether direct or indirect, on different RNA-binding proteins, including another ALS and FTLD relevant protein FUS. Improper RNA metabolism could have an important role in ALS and FTLD pathology.

Keywords: TDP-43, RNA-binding proteins, ALS, FTLD

A role for CK2 in dopamine signaling

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CK2, a heterotetrameric kinase consisting of two catalytic subunits and two regulatory subunits, is a major player in the regulation of cell proliferation and apoptosis. Despite high expression levels and activity, its role in the brain is poorly understood. CK2 substrates expressed in brain have been linked to a variety of brain disorders such as Parkinson's or Alzheimer's disease. Using biochemical and cellular approaches we have previously shown that CK2 negatively controls signaling via Gαs/Gαolf) coupled to dopamine D1 receptors. Pharmacological inhibition of CK2 or knockdown by RNAi lead to elevated cAMP levels in D1 receptor-activated neuroblastoma cells. Phosphorylation levels of protein kinase A (PKA) substrates were increased in the presence of CK2 inhibitors in mouse striatal slices.

We have now extended this work by studying a mouse conditional CK2α knockout (KO) line using Cre recombinase under the D1R-promoter. We tested these mice biochemically and behaviorally, and found that in striatal slices of KO mice phosphorylation levels of PKA substrates were increased to a greater extent in response to D1R agonist (SKF81297) than in control mice. KO mice exhibit elevated motor activity in the open field paradigm as well as enhanced exploratory behavior (as tested by novelty-suppressed feeding and novel object tests). The behavioral phenotypes are dependent on D1 dopamine receptor since a D1R antagonist (SCH23390) abolished them. Our data show that CK2 is an important modulator of dopamine signaling, a finding which could have implications for disorders like Parkinson's disease, schizophrenia or ADHD which are characterized by dopamine imbalance.

Keywords: dopamine, striatum, CK2, kinases, GPCR

Nuclear transport of TDP-43

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TDP-43 is a predominantly nuclear RNA-binding protein that forms inclusion bodies in frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). We have identified that TDP-43 is imported into the nucleus via the Karyopheirn beta 1 pathway and that knockdown of members of the pathway can lead to cytoplasmic accumulation of TDP-43. We have also observed that one of the members of the pathway, CAS (cell apoptosis susceptibility protein) is greatly reduced in the post-mortem brain tissue of patients who had FTLD, which may be one of the reasons for cytoplasmic TDP-43 accumulation in this disease. This finding suggests that the disturbance in the nuclear transport of TDP-43 could be one of the root causes of TDP-43 proteinopathies.

Keywords: TDP-43, nuclear transport, FTLD, ALS

Motor nerve regulation of myosin heavy chain I mRNA expression in mature and immature rat muscles

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Myosin heavy chain (MyHC) gene family consists of different isoforms, of which type I or slow MyHC is present predominantly in slow oxidative fibers, fast isoform IIa is in smaller, oxidative fast fibers, IIb is typically in the largest, fastest and most glycolytic fibers and IId falling between the last two. We examined the MyHC I mRNA levels in early postnatal and adult rat soleus, sternomastoideus (white and red part were analyzed separately) and extensor digitorum longus muscles. Furthermore, we investigated the MyHC I mRNA levels in denervated fast and slow-twitch muscles and also the effect of early reinnervation during muscle regeneration after injury. These muscles were injured by cutting all their blood vesels and by injecting a myotoxic anestethic bupivacaine. The MyHC I mRNA levels were determined by using the real-time PCR method. Our results confirm already reported difference between slow and fast muscles: MyHC I mRNA levels were highest in the soleus muscles with descending levels in sternomastoideus red part, extensor digitorum longus and white part of sternomastoideus muscles. There was no difference in MyHC I mRNA levels between adult denervated and control extensor digitorum longus muscles, but we found lower levels in denervated soleus muscles in comparison to the control ones. All non-innervated regenerating fast and slow muscles express very low MyHC I mRNA levels ten days of regeneration after injury, whereas in the innervated regenerating soleus (ten days after injury) there is a quick increase of MyHC mRNA, reaching the levels in the normal control muscles.

Keywords: skeletal muscle, myosin heavy chain I, regeneration

Regulation of parvalbumin mRNA expression in fast and slow rat muscle

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Parvalbumin is a small cytosolic Ca2+-binding protein highly expressed in mammalian fast- but not in slow-twitch muscles. It accelerates the process of muscle relaxation by binding the Ca2+ in the sarcoplasm before its pumping to the sarcoplasmic reticulum. Our aim was to examine the regulation of parvalbumin mRNA level in fast and slow rat muscles. First, we examined its expression in the rat fast extensor digitorum longus (EDL) and sternomastoideus muscles, slow soleus (SOL) muscle and in the diaphragm by using the real-time PCR method. Furthermore, we studied its levels in these muscles during early postnatal period. We assumed that the parvalbumin mRNA levels in muscles depend on the nerve. Finally, we checked whether the calcineurin signaling pathway might be responsible for the low parvalbumin expression in slow muscles. Our results confirmed that parvalbumin mRNA expression is high in muscles with high fast fibers type content and that its level in early postnatal development is significantly lower than that in adult muscles. The motor nerve inhibits the parvalbumin mRNA expression in the SOL muscles, while in the EDL muscles it allows its high level by activating this muscle with a phasic pattern of stimulation, whereas the chronic low-frequency muscle activation induced by electrical stimulation of the nerve decreased parvalbumin levels in fast muscles. Finally, the calcineurin signaling pathway is not involved in the reduction of parvalbumin expression in slow muscles.

Keywords: skeletal muscle, parvalbumin, denervation, electrical stimulation, calcineurin

Expression of neuroplastin is increased in hippocampal tissue affected by Alzheimer's neurodegeneration

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Neuroplastin, glycoprotein found in two splice isoforms (np65, np55), is a member of immunoglobulin superfamily including different cell adhesion molecules. Brain specific localization of np65 and its association with synaptic plasticity and long-term potentiation was found in rodent brain. Distribution of np65 in adult human brain has been described, however systematic data on its expression during developmental stages, maturation, aging and neurodegeneration are surprisingly lacking. Alzheimer's disease (AD) is characterized by up-regulated expression of plasticity molecules, particularly in hippocampus and entorhinal cortex, reflecting activation of compensatory mechanisms and reorganization of remaining cellular structures. In this study, the expression of neuroplastin (np65) was analyzed in human hippocampal tissue affected by Alzheimer's neurodegeneration. Paraffin-embedded sections of hippocampal tissue derived from 3 AD and 3 age- and gender-matched controls were analyzed by immunohistochemistry, using primary anti-neuroplastin antibody. Neuroplastin immunoreactivity pattern and intensity was additionally analyzed using ImageJ software. Results on distribution of neuroplastin immunoreactivity confirmed its extracellular localization in both control and AD hippocampal sections. The overall intensity of neuroplastin immunoreactivity was higher in AD than in control hippocampi, and was most notably expressed in neuronal population of dentate gyrus inner molecular layer; also, patch-like immunoreactivity was observed in stratum pyramidale of subiculum. Interestingly, unexpected intracellular localisation/accumulation of neuroplastin was detected in AD hippocampi, mostly in subiculum, which may indicate altered posttranslational modification and trafficking of neuroplastin molecule. We suggest that neuroplastin may serve as additional plasticity marker, as its expression is increased in AD neurodegeneration due to described reorganization and plasticity reactivation.

Keywords: neuroplastin, hippocampus, Alzheimer's disease

Delayed evolution of ischemic lesion and processes of cell death in TLR2 deficient mice

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Microglial activation following ischemic injury is associated with a strong induction of the innate immune receptors such as TLR2. Using in vivo imaging and the TLR2 reporter mice, we recently demonstrated that microglial activation/TLR2 response may persist several months after initial stroke. The aim of this study was to investigate the effects of microglial activation and the innate immune response, in particular TLR2 receptors on infarction size, cell apoptosis and its role in processes of post-ischemic inflammation in time dependent manner.

Unilateral transient focal cerebral ischemia was induced by middle cerebral artery occlusion (MCAO) during 1h followed by different reperfusion periods (3, 7 and 14 days after MCAO). In order to estimate size of ischemic lesion, 35µm cryostat sections were stained with cresyl violet. Immunohistological labelling was performed with TLR2, Iba1 and cleaved caspase-3 antibodies.

Marked induction of TLR2 signal in ischemic area was observed, compared to contralateral, non-ischemic area where no TLR2 signal was observed. Almost all of the TLR2 positive cells were colocalizing with Iba1 staining, suggesting that TLR2 receptors are mainly expressed on microglial cells. Assessment of infracted volume and number of apoptotic cells showed delayed evolution of ischemic lesion in TLR2KO mice compared to group of wild type mice.

These results show that evolution of ischemic lesion and processes of cell death are TLR2 dependant. Importantly, these processes act in time-dependent manner, and which must be considered in future planning of experiments which will try to explain effects of TLR2 deficiency on processes of post-ischemic inflammation.

Keywords: TLR2, stroke, MCAO, apoptosis, ischemic brain injury

SEMA3A regulates local axonal branching of GABAergic interneurons through fine regulation of cGMP level

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Different classes of GABAergic interneurons impose strong electrical regulation of their target cells by developing specific local axonal branching. Yet, the molecular mechanisms triggering this branching activity remain unclear. In cerebellar cortex, Basket interneurons form exuberant axonal branches at the axon initial segment (AIS) of Purkinje neurons and supposedly regulate their firing output. By using both in vitro and in vivo approaches, we showed that a member of the semaphorin family, SEMA3A, secreted by Purkinje neurons during local circuit formation induced basket axon branching at AIS. SEMA3A through activation of its receptors, directly regulates the level of cGMP in Basket interneurons. Indeed, SEMA3A modulates a soluble guanylate cyclase activity through tyrosine phosphorylation.

We propose a new transduction pathway in which SEMA3A, through direct regulation of cGMP level, induces local GABA axonal branching underlying synaptic tuning of their target Purkinje cell.

Keywords: SEMA3A, GABAergic interneuron, axonal branching, guanylate cyclase

Cluster analysis of AQP-4 in rat ALS model

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Astrocytic molecular pathway for water permeability in the blood-brain barrier (BBB), a complex glio-vascular system, is mediated by aquaporins (AQPs). We previously showed an overexpression of aquaporin-4 in the spinal cord in the end stage of amyotrophic lateral sclerosis (ALS) SOD1G93A rat model. Upon revealing, by electron microscopy (EM) with immunogold labelling, that the density of anti-AQP-4 appeared increased around blood vessels and that immunogold particles appeared grouped, we conducted a spatial analysis of AQP-4 clustering. Our current work considers the problem of density estimation and clustering of anti-AQP-4 gold in EM images around blood vessels in the SOD1G93A rat model for ALS. We have used several approaches such as density function estimators, hierarchical clustering and Markov chains to fully characterize particle distribution. By pre-clustering the data and analysing distances between particles we gathered sufficient data for Markov chain analysis, and then characterized distribution with post-analysis. Further optimization of the algorithm is still necessary, yet preliminary results have delivered a description of AQP-4 clustering. Identification of the clustering process should be important for understanding the mechanisms that govern water entry, which may be beneficial in preventing the disruption of BBB and in search for drugs that modulate clustering.

Keywords: aquaporin-4, ALS, cluster analysis, blood-brain barrier, astrocytes

Immunocytochemical localization of mammalian secreted phospholipases A2 in an experimental model of the in vitro innervated human muscle

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Mammalian secreted phospholipases A2 (sPLA2s) comprise a group of ten enzymes that catalyse hydrolysis of the sn-2 ester bond of phospholipids to generate free fatty acids and lysophospholipids. Their diversity in structure, enzymatic properties, and tissue distribution argue for a wide variety of physiological and pathophysiological functions. Recently, certain mammalian sPLA2s were found localised in the peripheral nervous tissues, where their counterparts, isolated from snake venoms, exert strong toxic action.

The aim of our study was to validate the experimental model of the in vitro innervated human muscle, as a system to study the molecular mechanism of mammalian and/or snake neurotoxic sPLA2s. Since it consists not only of the motor neurons and myotubes but also of supporting glial cells, which are essential for the normal development of the motor neurons, neuromuscular junctions differentiate and become functional in this system. Using this experimental model, we immunocytochemically analysed different groups of mammalian sPLA2s. We also applied directly fluorescently labelled mutants of sPLA2s to investigate their presence and localization. Our preliminary results suggest that the experimental model of the in vitro innervated human muscle is more complex than the neuronal cell culture systems, but valuable and useful for the study of role and function of sPLA2s in the peripheral nervous system.

Keywords: secreted phospholipase A2, neuromuscular junctions, experimental model

Downregulation of miR-195 via Cyclosporin A suppresses the growth of human glioblastoma cells

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Cyclosporin A (CsA) is a powerful immunosuppressive agent. MicroRNAs (miRNAs) are a class of recently discovered non-coding RNA genes that post-transcriptionally regulate gene expression. It is aimed to investigate the effects of CsA on the potential 88 miRNA expression changes in glioma cells-U87-MG.

U87-MG cell line grown in BIOAMF-1 medium supplemented with 10 000 U/ml penicillin, 10 mg/ml streptomycin, 2 mM L-glutamine was maintained at a density of 5 X 105 cells/ml. CsA was used in treatments of 10, 30 and 60 µM. Cytotoxic assays and determination of IC50 dose of CsA in U87-MG cells were performed. The cell group that was not treated by any agent was approved of the control group. Relative quantitation of 88 miRNAs was measured by Light Cycler 480 Real Time PCR. SNORD48, SNORD47, SNORD44, U6 were used as human endogenous controls. The fold changes of miRNAs determined and alterations in the miRNA expressions were compared with CsA treated and CsA- free U87-MG glioma cells.

In U87-MG cells treated with CsA (10 µM), 3 of 88 human miRNAs were up-regulated and 40 were down-regulated as detected with the miRNA array compared with control group. It is found that expression levels of several miRNAs, in particular, miRNA-195, was significantly decreased in CsA treated U87MG cells.

The study can provide important roles of miR-195 in GBM pathogenesis and in the molecular etiology of GBM. Rather than knockdown of miR-195 for moderate cell killing effect, treatment with CsA could be more effective especially on temozolomide resistant cells.

Keywords: Cyclosporin A, U87-MG, miR-195

Ganglioside composition and structure analysis in human dysembryoplastic neuroepithelial tumor

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Gangliosides, a complex group of sialylated glycosphingolipids, are plasma membrane components involved in cell-cell and cell-matrix interactions and in modulation of cell signalling. They are especially abundant in the mammalian brain tissue and they participate in numerous physiological processes. Glycosphingolipid metabolism abnormalities are implicated in the initial oncogenic transformation and tumor progression, resulting in different ganglioside expression in tumor, compared with normal brain tissues. Elucidating the role of tumor-associated gangliosides in transformed cells presents a significant factor in current cancer research.

Compositional and structural analysis of ganglioside mixture from dysembryoplastic neuroepithelial tumor (DNET) was performed. Total gangliosides were isolated and purified as native mixtures from tissue homogenates and spectrophotometrically quantified. HPTLC was used for compositional analysis. MS screening and sequencing analyses were carried out on a High Capacity Ion Trap Ultra, coupled with fully automated chip-based nanoelectrospray NanoMate robot. The total GG content determined in tumor (DNET) sample was approximately 10 times lower than in the normal adult human brain. The HPTLC pattern of tumor was highly distinctive from normal brain tissue. MS screening of the analyzed tumor sample revealed complex ganglioside composition, confirming the presence of GG species previously reported as tumor-associated ones (O-acetylated GD3, di-O-Ac-GD3). Unusual minor species such as O-Ac- and di-O-Ac-GD1, GT1 and GQ1, Fuc-GQ1, GalNAc-GT2, GalNAc-GT1, O-Ac-GP1 and GH1 were also detected. These results confirm the applied analytical strategies as a powerful GG fingerprinting tool for identification of biomarker species related to normal vs. pathological changes in the brain.

Keywords: gangliosides, human brain, brain tumors, HPTLC, mass spectrometry

Distribution of extracellular matrix molecules in a fetal and neonatal human brain

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In order to analyze extracellular matrix (ECM)composition in a developing human brain we used postmortem brain tissue, age ranged from 12 to 42 weeks post conception (WPC). To reveal carbohydrate component of the ECM we used biotinilated lectins: WFA, SNA, as well as colloidal iron staining, and we used immunohistochemical labeling to show proteins: chondroitin-sulphate proteoglycans (CS-56), fibronectin, laminin and syndecan.

Our findings indicate that during mid-gestation GAGs in the subplate displayed a gradient of expression with an initially higher expression in the deep subplate, which subsequently shifted to a higher expression in the superficial subplate after 22 PCW. During the late fetal and neonatal period, the expression of SNA-binding glycoconjugates shifted within the cortical plate and displayed differential expression within the immature neocortical layers. During the mid-fetal period, the CS-56 expression was present in the marginal zone and at the interface between the cortical plate and the subplate. The expression of ECM-fibronectin during mid-gestation was most prominent in the deep subplate.

In conclusion, the pattern of distribution of ECM molecules reflected the formation of transient fetal zones in the telencephalic wall and the initial differentiation of the cortical layers. The timing and relative abundance of the expression of ECM molecules within the subplate suggests that they may be involved in the development of thalamocortical and long cortico-cortical pathways, as well as the perinatal formation of short cortico-cortical Meynert's U-fibers. Therefore, disturbances in their expression may have a significant role in the pathogenesis of various developmental brain disorders.

Keywords: subplate, cortico-cortical pathways, glycoconjugates, perinatal brain

An immunological insight into the hypothalamic proline-rich polypeptide PRP-1 protective activity in vivo against methicillin-resistant Staphylococcus aureus infection

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Proline-rich polypeptides isolated from neurohypophyseal and hypothalamic neurosecretory granules represent a new family of hypothalamic neuropeptides which are synthesized in the form of a common precursor protein − neurophysin-vasopressin-associated glycoprotein (NVAG) by genetically determined mechanisms and released from the precursor by proteolysis during axonal transport. One of these peptides, a 15 amino acids PRP-1 or Galarmin (AGAPEPAEPAQPGVY) possess a broad-spectrum of biological activities including antibacterial, antitumor, immunomodulatory and neuroprotective properties. PRP-1 is a regulator of humoral and cellular immunity, thymocyte differentiation, and myelopoiesis. Systematic administration of PRP-1 can prevent neurodegeneration in hippocampus induced by amyloid peptide Aβ 25-35 and protects from aluminum neurotoxicity causing Alzheimer-like disease. PRP-1 was shown to be also powerful antibacterial agents in vivo against such pathogens as Bacillus antracis, Clostrdium perfringens and Micobacterium tuberculosis. Our data indicates strong prophylactic activity of PRP-1 against methicillin resistant S.aureus (MRSA). Recent increase of MRSA strains at large hospitals as well as community settings (community associated) started to pose great difficulty in selecting antimicrobial agents. We have shown that PRP-1 could fully protect mice against a lethal infection of MRSA injected 24h before infection and 1h post infection period at a concentration of 1 μg per mice, and its higher concentrations (5 and 10 μg) protect animals against MRSA, even when injected simoultaniously with the bacteria. It was demonstrated that the protective activity of PRP-1 is not due to a direct effect on bacteria, but rather on the host response to infection. We could show that PRP-1 significantly increased the level of pro-inflammatory cytokine IL-6, IL-8 and modulate the expression of serum immunoglobulins IgA, IgM and IgG. The hematological effect (complete blood count) under PRP-1 influence was revealed as well. Received data demonstrate that PRP-1 plays regulatory role in immune response modulating process during MRSA infection.

Activity of SKa-31 against seizure-like events in rat organotypic hippocampal slice cultures

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sK-channel plays role in regulating neuronal excitability, thus it might be of interest to suppress seizure in epileptic patients. Based on role of sK-channel we aimed to test enhancer of sK-channel in rat organotypic hippocampal slice cultures (OHSCs) against seizure-like events (SLEs) induced by 4-aminopyridine or Mg^{2+} free ACSF. Slice culture were prepared from p5-p7 rat pups [1] and incubated with maintained condition i.e. 5% CO_2, 36.5°C. Cultures were fed every alternate day, whereas 7-14 days old cultures were used for electrophysiological recordings using interface setup.

SKa-31 (sK-channel enhancer) at dose of 150 μM completely suppressed seizure-like events, standard antiepileptic drugs sodium valporate (2 mM) and carbamazepine (100 μM) were also blocked SLEs induced by 4-AP. All drugs were unable to block status or late recurrent discharges induced by Mg-free ACSF. Data suggest sK channel enhancer has potential to suppress acute seizures but not pharmacoresistant epileptic seizures.

Reference:

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Effect of neuraminidase-inhibition on synaptic plasticity in rat hippocampus

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Neuraminidase (NEU) affects neuronal and network activity by controlling of sialylation of the extracellular membrane. Treatment with NEU lead to significant depolarizing shift of the voltage-gated sodium channel activation and inactivation and as a result increase of action potential threshold. At the network level NEU exerts powerful anticonvulsive action in vitro and in vivo. On the other hand blockage of endogenous NEU with N-acetyl-2,3-dehydro-2-deoxyneuraminic acid (NADNA) results in aggravation hippocampal seizures in vitro and in vivo and leads to synaptogenesis In the present work we investigated effect of blockage of endogenous NEU on short and long term plasticity in the CA1 region of the rat hippocampus.

Temporal lobe slices which contain neocortical areas, the entorhinal cortex, subiculum and hippocampus, were prepared from Wistar rats aged postnatal day 19-21. Evoked synaptic responses were elicited through a bipolar electrode placed in the Schaffer collateral-commossural pathway. Extracellular recordings were obtained from pyramidal cell layer and stratum radiatum CA1 regions.

Temporal lobe slices were treated with NADNA for 2 hours. We show that blockade of NEU leads to an increase of augmentation in CA1 region of rat hippocampus. In the majority of cases stimulation of Schaffer collaterals leads to a very short-term potentiation in control slices. In the most of NADNA-treated slices long-term potentiation was observed after the delivering of the same stimulus. Also we evaluated effect of pretreatment with NADNA on the short-term synaptic depression of field postsynaptic potentials (15 stimuli within a 50-Hz train). The main difference between NADNA-treated and untreated slices was in manifestation of first and second responses. Most slices pretreated with NADNA show depression after first stimulus, while the majority of control slices display facilitation.

The present data show that NEU-blockage affect synaptic plasticity in rat hippocampus. Synapses with increased probability of release tend to display depression, presumably because the pool of vesicles available for release becomes depleted after initial successful release. We propose that in NADNA-treated slices enhancement of transmitter release probability following synaptic activation can be one of the possible mechanisms of increased synaptic plasticity in NADNA treated slices.

Alcohol self-administration in the Sprague-Dawley rat: the role of the glutamate

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Alcohol addiction is a chonic disease characterized by an inability to control drinking even after long periods of abstinence. Relapse to alcohol-seeking can be modeled in animals using the extinction reinstatement paradigm. This paradigm involves training animals to respond for alcohol reinforcement in a standard two-lever operant chamber. The operant response is then extinguished and reinstated. Here we sought to determine the most efficient method for inducing high levels of unsweetened alcohol consumption in the operant chamber as well as consistent reinstatement behavior. We compared two methods: one method which trained animals using 12 overnight operant sessions followed by 45-minute sessions on alternating days for 26 sessions (designated the 12Hr group) and a second method which trained animals in the operant chamber only during daily 45-min sessions for 26 sessions (designated the Daily group). Presses on the alcohol-paired lever resulted in delivery of alcohol (20% v/v) into a dipper tray and presentation of discreet cues (light+tone complex). Both groups had been previously trained to consume unsweetened ethanol (20% v/v) in the home cage using the Intermittent-access Drinking Paradigm (IDP; Simms et al., 2008) for either 5 sessions (12Hr) or 12 sessions (Daily). A third group only consumed alcohol using the IDP and were not trained to self-administer alcohol in the operant chamber (designated the IDP group). Our results show no significant difference in total alcohol consumption between the three groups (12Hr, Daily, and IDP). The 12Hr and Daily groups did not differ in operant responding for alcohol reinforcement and both groups showed similar responding during extinction training. While there were no significant differences in reinstatement behavior between the two groups in response to either cues or yohimbine (2 mg/kg), there was a trend for higher lever pressing in the Daily group. Only the Daily group showed an attenuation of yohimbine primed reinstatement following N-acetylcysteine (NAC;100 mg/kg) treatment. These results indicate that training Sprague-Dawley rats to self-administer alcohol in the operant chamber can be accomplished using daily training sessions without 12 hour operant sessions. Furthermore, NAC inhibits yohimbine-primed reinstatement in animals trained to self-administer alcohol using the Daily method. Western blotting was performed on tissue from the nucleus accumbens of animals in the IDP group following both 24 hr and 7 days withdrawal. The expression of the glutamate transporter GLT-1 was significantly increased at both withdrawal times. Taken together, these results indicate that the glutamate transmitter system is altered by alcohol self-administration.

Effects of perinatal treatments with 5-hydroxytryptophan and tranylcypromine on serotonin-related behavior in adult rats

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Due to its dual role in brain development and function, serotonin (5HT) mediates many essential behaviors common to all mammals, including exploratory and anxiety-related behavior. The aim of this study was to investigate serotonin-related behavior in adult animals after exposing their developing brains to increased 5HT concentrations using two alternative approaches: by primarily increasing 5HT concentrations in the periphery through treatment with the immediate 5HT precursor, or by altering serotonin metabolism in the brain and periphery through treatment with a non-selective MAO inhibitor. Wistar rats were treated subcutaneously with 5-hydroxytryptophan (5HTP, 25 mg/kg), tranylcypromine (TCP, 2 mg/kg), or saline, from gestational day 13 to post-natal day 21. Adult animals were tested for locomotor activity, exploration and anxiety, social choice and reaction to stressful stimulus. There were no differences in locomotor activity among the groups. 5HTP-treated rats showed significantly increased exploratory activity in a hole-board and spent more time exploring an inanimate object in the social choice test. TCP-treated rats displayed reduced thigmotactic anxiety in a hole-board, reduced freezing behavior in response to stressful stimulus, and decreased social anxiety reflected in more time spent in exploring a conspecific in the social choice test. The results indicate that perinatal treatments with 5HTP and TCP have indeed affected brain development, altering so serotonin-related behavior in adult rats in a way which corresponds to decreased 5HT function: increased exploratory and decreased anxiety-related behavior.

Keywords: serotonin, exploratory behavior, anxiety-related behavior, 5-hydroxytryptamine, tranylcypromine, rats

The effect of acute and subchronic folic acid administration on electroencephalographic characteristics of homocisteine induced epilepsy

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Generally folic acid has been regarded as non-toxic. Long ago folic acid developed reputation for provoking seizures, but its toxicity is controversial.

The aim of the present study was to examine the effect of acute and subchronic folic acid administration on electroencephalographic (EEG) characteristics of DL homocysteine thiolactone induced seizures in adult rats.

Adult male Wistar rats were divided into following groups: 1. Control (C); 2. DL-homocysteine thiolactone 8 mmol/kg, i.p.(H); 3. Acute folic acid administration i.p. in doses: 5, 10 and 15 mg/kg, (F5, F10 and F15); 4. F 30 min prior to H (F5H; F10H; and F15H); 5. Subchronic F i.p. in doses: 5 and 15 mg/kg, (CF5, CF15) for 7 days; and 6. CF + H (CF5H, CF15H). For EEG recordings, three gold-plated electrodes were implanted into the skull. The observational period (120 min) was divided into eight 15 min intervals and mean total power spectral density (PSD) was calculated (fast Fourier transform method). Total median number and duration of spike-wave discharges (SWD) was calculated.

Dissociation between EEG pattern and motor phenomena was common to all experimental recordings. Acute F significantly decreased while subchronic F significantly increased PSD dose-dependently, comparing to H. Both acute and subchronic F administration completely abolished the occurrence of SWD during observational period.

Our findings suggest that acute administration of F has antiepilepetic while subchronic H exerts mild proconvulsive effect on H induced seizures in adult rats.

Keywords: folic acid, DL-homocysteine thiolactone, epilepsy, power spectral density, spike-wave discharges, rats

Non-ACTH-mediated glucocorticoid secretion regulation of the adrenal cortex

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The maintenance of life depends on the capacity of the body to sustain its homeostasis and the hypothalamo-pituitary-adrenal axis is a key element of it. According to the textbooks adrenocorticotropin (ACTH) stimulates the adrenal cortex to release glucocorticoids into the blood. However several data suggest an ACTH-independent glucocorticoid secretion. Our aim was to find other regulatory molecule(s). As during stress catecholamines are also released from the adrenal medulla, we proposed that adrenalin could be such a molecule.

To test our hypothesis we used a model organism, the 10 days old vasopressin deficient Brattleboro rat, as previously 24h maternal separation was able to induce glucocorticoid elevation without ACTH peak in them. In 4h-fasted pups 90 min after an ip injection of 3NE/kg rapid insulin we could detect hypoglycaemia together with an activation of the stress axis. In vasopressin-deficient pups the presence of ACTH-independent glucocorticoid secretion was reproduced. Pretreatment with a β-adrenerg antagonist (15 min, 2.5mg/kg propranolol) reduced the hypoglycaemia-induced glucocorticoid elevation without affecting the ACTH levels. In vitro the glucocorticoid secretion of the adrenal gland of a normal pup was enhanced by ACTH (10-10M) and propranolol (10-5M) treatment reduced both the basal and ACTH-induced secretion.

Our results support the view, that adrenaline might stimulate the glucocorticoid secretion from the adrenocortical cells. As the effect of the antagonist was not complette we can assume that other regulatory molecules might have an impact, too.

Keywords: HPA axis, ACTH, corticosterone, adrenaline, Brattleboro, hypoglycaemia, propranolol

Severity of lindane-induced seizures: alteration by 7-nitroindazole

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7-nitroindazole (7-NI) is a selective inhibitor of neuronal nitric oxide synthaze (nNOS), an enzyme responsible for production of gaseous neurotransmitter NO, recently extensively studied as mediator in excitability of central nervous system. Lindane is environmentaly persistant scabicide and pesticide. One of the first manifestations of its neurotoxic effects are seizures. The aim of this study was to investigate, using 7-NI, the effects of nNOS inhibition on behavioral manifestations of lindane–induced seizures.

Male Wistar albino rats of adult age were intraperitoneally (i.p.) treated with lindane 4 mg/kg and observed for seizure behavioral manifestations during next 30 min. Increasing doses of 7-NI (25, 50 and 75 mg/kg, i.p.) or saline were injected 30 min prior to lindane administration. Seizure behavior was assessed by latency time to seizure onset and its severity assessed by descriptive rating scale with 4 defined grades.

It was shown that 7 -NI administered 30 minutes before lindane significantly increased seizure severity and decreased latency time.

Results of this study indicate that systemic administration of 7-NI, a selective nNOS inhibitor, potentiates lindane- induced seizures in rats.

Keywords: lindane, seizures, nNOS, 7-nitroindazole, rats

A simple mathematical model accounts for the reactive electrocortical burst-suppression behavior during anesthetic coma

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Burst-suppression (BS) electrocortical pattern, consisting of "bursts" of activity on a suppressed/isoelectric background, accompanies deep comatose states. When the "spontaneous" bursting behavior can be modulated by external stimuli, the BS pattern is characterized as "reactive" and it is considered a marker of good prognosis for coma recovery. Nevertheless, current methods employed to quantify the functional cortical network impairment during BS do not account for its reactivity.

We investigated the reactive bursting behavior in response to visual stimulation (super-bright flashes delivered at 0.5 Hz in 1 min epochs) during chloral hydrate anesthetic coma in Wistar rats.

We found that during visual stimulation most if not all bursts occurred 200-300 ms after the flashes following a stereotypical behavior: (1) after the onset of stimulation increasingly more flashes failed to trigger bursts and (2) after the offset of stimulation the recovery of "spontaneous" bursting was delayed. The magnitude of these effects increased with the depth of anesthesia as reflected by the rate of "spontaneous" bursting. This complex reactive behavior could be simulated using a simple mathematical model considering that each burst causes a cumulative increase in the threshold for burst generation (TBG), followed by an exponential recovery with a time constant (Tau) proportional to the depth of anesthesia.

Our model suggests that during BS stimuli of various strengths compete to overcome the TBG, which depends on both the anesthetic depth and previous bursting history. We propose Tau as a reliable biomarker for monitoring the extent of functional connectivity impairment in the comatose brain.

Keywords: burst-suppression, coma, anesthesia, rat, visual stimulation

Decreasing connectivity and functional network size in the CA3 region of thick hippocampal slices, reduces sharp-wave incidence

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Hippocampal sharp-waves (SPWs) are generated in the CA3 area, and propagate to the CA1 subfield via Schaffer-collaterals. Here we examined the mechanism of SPW initiation in vitro. Our working hypothesis was that when adequate number of pyramidal cells are concurrently firing, activity starts to build up in the recurrent excitatory network of the hippocampus. This model predicts that the larger the network is, the more frequent and regularly timed the SPW episodes are. SPWs are spontaneously present in thick (600µm), submerged in vitro mouse hippocampal slices, superfused on both sides with normal excitability ACSF. We recorded local field-potentials from these slices, and tested the dependence of the frequency and the interevent interval distribution of SPWs as a function of virtual network size. To reduce functional network size and connectivity, axonal conduction was partially blocked in the recurrent collateral system of the CA3, by tetrodotoxin (TTX) injection via a glass capillary.

Injecting TTX reversibly decreased sharp-wave frequency (or stopped SPW generation if large injections were applied), and increased interevent interval variability without affecting the nature and other parameters of the SPWs. This experiment reinforces the model that SPW initiation is a stochastic process, and at the same time reject models suggesting that the length of inter-SPW intervals are set by refractory or depletion mechanisms at the neuronal and/or network level.

Keywords: sharp-waves, hippocampus

The influence of dietary restriction on phytosterol levels in the aging rat brain

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Phytosterols are lipid compounds structurally analogous to cholesterol, with the same basic functions in plants as cholesterol has in animals. Cholesterol has a crucial role in development and maintenance of neural plasticity and function. Disturbance in cholesterol metabolism, along with aging, represent an important risk factor for AD. Phytosterols have the potential to reduce cholesterol absorption by 30% to 50%.

Considering the crucial role of cholesterol and its metabolism in the brain, the aim of this study was to define the effect of long term dietary restriction (DR) on the content of plant sterols in the aging rat brain.

The experiments were performed on 3-, 12-, and 24-month-old male Wistar rats fed ad libitum (AL) or exposed to long term DR (100% EOD) starting from 3 months of age. Levels of brassicasterol, campesterol, stigmasterol and sitosterol, the most abundant plant sterols, in rat cortex and hippocampus were determined using gas chromatography-mass spectrometry (GC-MS).

These results showed that plant sterol levels were increased during aging in both cortex and hippocampus. DR had no influence on brassicasterol content in both cortex and hippocampus. Regarding cortex, DR maintained control levels of campesterol, stigmasterol and sitosterol during aging. In hippocampus, DR influenced campesterol and sitosterol levels in 24-month-old animals. DR affected stigmasterol content starting from 12 months of age.

Obtained results showed that DR counteracted age-induced accumulation of phytosterols in the brain, but potential beneficial role of DR in this case had to be further elucidated.

Keywords: phytosterols, brain, aging, dietary restriction

Laminar distribution of the slow oscillation in rat somatosensory cortex under anesthesia

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The slow oscillation or slow-wave activity (SWA) is a brain rhythm that emerges during natural slow-wave sleep and in narcosis, and is present over the whole cortical mantle. SWA is characterized by rhythmic alternation of two phases: the "up-state" with strong synaptic activity and cell discharging due to membrane depolarization and the hyperpolarized "down-state" with neuronal silence. The exact mechanisms underlying the SWA are still unclear, but recent evidence suggests that the generation of the slow oscillation in humans is of supragranular origin. To compare human results to animal data, we recorded the activity from the trunk region of the rat somatosensory cortex with 24-contact laminar multielectrodes during ketamine-xylasine (KX) anesthesia. KX models the natural SWA by producing a regular, continuous slow oscillation with 1.5 Hz peak frequency. Local field potentials (LFP), LFP gradients (LFPg), current-source density(CSD) and multiple-unit activity(MUA) were extracted from the wideband recordings. Up-state locked averages showed strong inward synaptic/trans-membrane currents in supragranular and granular layers, surrounded by two current sources near the cortical surface and in infragranular layers. This pattern was reversed during down-states. In the active state, MUA was strongest in layer V and diminished significantly in layers I, II and VI. Our results suggest that the generator mechanism of SWA in rodents is partially similar to that of humans, since the most impressive current sink is located superficially in both cases. However, the existence of substantial outward currents in deep layers and the infragranular MUA maximum may reveal possible differences between the two conditions.

Keywords: slow oscillation, slow-wave activity, local field potential, current source density, multiple-unit activity

Evaluation of a Bayesian model of pain modulation and placebo effect

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Pain is a highly subjective experience and several cognitive factors can modulate pain perception. Research in this field is mainly focused on neuronal circuits and neurobiological processes involved in pain perception and few studies have proposed theoretical models for better understanding the influence of these factors.

The aim of this study was to evaluate a Bayesian theoretical model of pain perception. In particular, the model we propose can describe the changes in subject's pain scoring after a conditioning session in a placebo experimental paradigm.

To validate our model, two groups of volunteers underwent two different experiments in which analgesic placebo effect were induced by a conditioning training with high- and low-intensity painful stimuli, paired with two visual cues. Mechanical stimulation was used in the first experiment, electrical stimuli was applied in the second one. Pain perception was assessed by means of a Visual Analog Scale (VAS). We found that conditioning could modulate pain sensation as demonstrated by the difference in subjects' pain scoring in the testing session, in which the same stimulus intensity was applied with both cues. Moreover, the results demonstrated that the stimulus-VAS curve was modified after the conditioning even in the absence of any visual cue as predicted by our model.

These findings support the validity of our Bayesian model as a possible new theoretical framework for understanding the role of cognitive factors in pain modulation.

Keywords: pain, placebo, Bayes

Pain perception and placebo analgesia as a Bayesian probabilistic inference

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Clinical and research evidence showed that cognitive and emotional factors can modulate pain perception, and that pain modulation is affected by past experience. This suggests that pain perception may result from the integration of different pieces of information, and that learning my shape such a process of integration. Bayesian decision theory (BDT) is a logical and mathematical framework which can account for such processes. BDT succeeded in modeling different biological processes, such as perceptive integration and motor choice strategies. BDT models are good not only in fitting the data, but they appear also to reflect the strategies used by biological systems themselves. Aim of this study was to investigate a theoretical model of pain using BDT, which may be of great value in explaining relevant features of nociception, such as the analgesia observed in danger or under high motivation, and pain modulation induced by emotions or placebo. Indeed placebo analgesia has been used to study my probabilistic model of pain and its modulation. Integrating different pieces of information and past experience, the model successfully predicts experimental observations of placebo analgesia. Moreover, it makes a spectrum of predictions about never investigated aspects of pain modulation. Such results suggest that placebo analgesia is not a fault of the system, but the most probable estimate given the available data and experience; and that a Bayesian probabilistic approach can be a valuable tool to understand nociceptive processes and the meaning of many related phenomena, so providing interesting insights for further understandings and practical outcomes.

Keywords: pain, placebo, Bayes

Synaptotagmins 4 and 7 are not involved in the striatal vesicular transport of neuropeptides substance P and enkephalin

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Synaptotagmins (Syts) are proteins thought to be essential for regulated membrane trafficking in the brain. Specifically, Syt 4 and 7 are implicated in the regulated exocytosis of dense-core vesicles in neuroendocrine cells, but in neurons that was not shown before. It is known that neuropeptides substance P and enkephalin are abundantly synthesized in the striatum and transported/exocytosed to their target areas by dense-core vesicles. We therefore aimed to examine the involvement of Syt 7 and Syt 4 in the transport of striatal dense-core vesicles containing substance P or enkephalin. In this study we performed double immunofluorescence staining for Syt 4 and Syt 7 proteins with substance P or enkephalin in the striatum of intact rats. Our results show no co-localization of immunosignals for Syt 4 or Syt 7 with immunosignals for substance P or enkephalin. We conclude that Syt 4 and Syt 7 are not present on substance P and enkephalin containing vesicles suggesting that they are probably not involved in the trafficking of respective neuropeptides in the striatum of intact rats.

Keywords: Synaptotagmin 4, Synaptotagmin 7, substance P, enkephalin, immunofluorescence

Prenatal and early postnatal development of modular organization in the human striatum

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The mammalian neostriatum is organized as heterogeneous mosaic of various compartments that can be distinguished according their times of neurogenesis, anatomical connections and distribution of neuroactive substances. It is interesting that histochemical and cytoarchitectonical inhomogenties described in the developing brain are more pronounced than the ones described in the adult brain. However, exact relationship between fetal and adult modular organization of the striatum remains unclear especially in humans, where systematic study of development of striatal compartments is still missing. According to differences observed in prenatal and early postnatal human material stained with Nissl, acetyl-cholinesterase (AChE) histochemistry and several immunohistochemical markers we can distinguish four developmental periods. In a period from 10-14 postovulatory weeks (POW) first inhomogenities in the human fetal striatum are observed only by means of AChE. Cytoarchitectonical cell islands are first recognized on Nissl sections at 15 POW and are matching the islands of increased NeuN, tyrosine hydroxilase (TH) and synaptophysin immunoreactivity. 20-28 POW period represents a peak in fetal modular organization of the striatum that is characterized by appearance of cell free perimeters around islands. At 27-28 POW MAP-2 staining completely defines island compartment whereas synaptophysin immunostaining starts to shows uniform distribution. This pattern is present in developing human striatum until 3rd postnatal month when MAP-2, TH and AchE positive islands start to disappear in the putamen probably due to the increase in matrix staining.

Keywords: striatum, human development, dopamine islands, matrix, AchE, MAP-2

Chronic fluoxetine treatment has antidepressant effect in female but not in male mice behavior in forced swim test

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Numerous studies have reported gender differences in the prevalence of major depressive disorder in humans. Most previous studies have been done in men and in male rodents, but there are fewer behavioral observation studies investigating effects of the antidepressant selective serotonin reuptake inhibitors (SSRIs) in female rats and even less in female mice. It is known that fluoxetine and other SSRIs decrease immobility time in the forced swim test (FST) and increase active swimming time in male rats (antidepressant effect).

In the present study we examined sex differences in FST in mice treated chronically with fluoxetine. The C57BL/6J mice were divided into four socially housed groups: control males and females, treated males and females. Treatment length was at least 14 days with 10mg/kg/day of fluoxetine in drinking water prior to behavioral assessment that involved elevated plus maze, open field test, social recognition test and FST. Only females in diestrus were tested to avoid possible differences in behavior due to hormonal effects. Fluoxetine did not have major impact on any of the behavioral measures evaluated in the mice tested, although there was indication of difference in FST in response to fluoxetine treatment between males and females, and this difference in mice differ from previous studies in rats. Therefore species and sex have to be taken in to account when assessing the effects of anti-depressants.

Keywords: mice, behavior, sex difference, SSRI, fluoxetine

Neural stem cells-enriched tubulization improves anatomical and functional restoration of severed rat sciatic nerve

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Tubulization of the severed peripheral nerve has recently emerged as a promising strategy to promote nerve reconstruction and functional restoration of the affected limb. Interestingly, neurotubules can be loaded with growth-promoting factors or cells, which may speed-up and/or optimize the final outcome, however, only relatively scant evidence is available. In the present study, we have investigated this possibility after the formation of a 5-mm gap and tubulization of the rat sciatic nerve with a cell-compatible, biodegradable PLCL (poly DL-lactide-ε-caprolactone, Neurolac®) copolyestere tube filled with either cultured human neural progenitors or with their conditioned medium. The in vivo analyses included also groups of animals subjected to direct suturing of the transected sciatic nerve, animals with a gap and no implant, and animals implanted with unloaded PLCL tube. Starting from one week post-surgery the animals underwent weekly evaluation of the lesion and treatment effects, by analysing indexes of sensory-motor function of the lesioned limb, as compared to the intact contralateral side. At the end of the experimental period (5 months) histochemical and retrograde fluorescent tract-tracing procedures were carried out to assess the anatomical and functional integrity of the nerve. The results showed a better functional recovery in the animals with no gap and direct suturing and in those implanted with the cell- or medium-loaded tube compared to the other groups. Thus, tubulization associated with local supply of growth-promoting factors may represent a viable strategy for functional nerve reconstruction, but further detailed analyses are needed before traslating it into clinical use.

Keywords: sciatic nerve transection, tubulization, neural stem cell, implantation, recovery

Tangential migration in the human telencephalon during second half of gestation

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The source of cortical GABA-ergic neurons has unique features in human and non-human primates (Petanjek et. al, Front Neuroanatom 2009;3:26.). In contrast to other mammals, where vast majority if not all are born in ganglionic eminence (GE) and migrate to dorsal telencephalon, in primates about 2/3 of cortical GABA-ergic neurons originate from the local proliferative zones.

We have analyzed frontal sectioned slices of the human fetal telencephalon during second half of gestation impregnated by Golgi method. During the period from 22 to 26 postconceptional weeks (pcw) numerous nonradially oriented migratory like unipolar cells have been observed in telencephalon, most densely packed in the subventricular and intermediate zones. Also, a large and very densely packed stream of migratory like cells leaving well pronounced GE in direction of dorsal telencephalon was observed at cortico-striatal border. At stage of 32-36 pcw there was still a significant number of nonradially migratory like cells in the dorsal telencephalon, while the GE decreased in size, but was clearly distinguishable. Also, a stream of migratory like cells was extending in ventro-dorsal direction and continuing out of the GE at the position of cortico-striatal border. During whole period many nonradially oriented migratory like cells in dorsal telencephalon, as in the stream leaving GE, were MAP-2 and calretinin positive.

These observations suggest that in the human brain significant production of subpopulations of cortical GABA-ergic neurons occurred much beyond proliferative period for principal glutamatergic neurons and extend even up to early postnatal period.

Keywords: interneurons, neurogenesis, tangential migration, ganglionic eminence, outer subventricular zone, MAP-2, calretinin

The effect of single acute cocaine exposure on the local network activity of PFC neurones in mice in vivo and in vitro as revealed by optogenetic methods

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Drugs of abuse modify cortical circuitry on the short and long term and lead to catastrophic effects at individual and social level. The effects of cocaine in the physiology of the prefrontal cortex are still poorly understood. We use optogenetic and pharmacological methods in vivo and in vitro to reveal local effects.

We induced expression of channelrodopsin-2 in PV fast-spiking interneurons with AAV virus injection in PV-Cre knock-in mice. Upon expression of the protein we identified and stimulated PV+ neurons with 470 nm laser impulses. Using 1 Hz or 40 Hz stimulation, we performed single-unit, multiunit and field-recordings in vivo, and whole cell and field recordings in vitro in the mPFC of mice. Upon an acute IP injection of cocaine (15mg/kg) we detected in the mPFC in vivo: 1) a change in the dynamics of the inhibitory effects on the local network 2) a change in the variance of the LFP signal 3) characteristic changes in the frequency power spectrum of the responses. Whole cell recordings in vitro revealed that acute cocaine administration (3 μm) elicited a decrease in the amplitude and charge of the eIPSCs. With pharmacological testing we traced the origin of these changes.

Our results suggest that a single exposure to cocaine causes characteristic changes in cortical network activity acting on local dopaminergic mechanisms. These changes, related to the change in the higher frequency oscillatory activity may underlie the behavioral effects of cocaine and lead to longer lasting neuroadaptation effects.

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Keywords: cocaine, optogenetics, gamma, LFP, whole cell

Phase of spike coding of sounds in the hippocampus

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Encoding of sensory events in hippocampus is crucial for episodic memory. Odors, sounds and textures cause firing rate changes in hippocampal neurons, which are typically measured on the order of hundreds of milliseconds. Yet, fine temporal precision of neuronal activity is crucial for associative plasticity. How sensory events are encoded in the temporal patterns of spikes was the question of our study.

Four stimuli two-alternative forced choice discrimination task was designed to disentangle neural activity related to sounds and place. Sound-specific changes in the preferred phase of firing and alteration of phase-locking strength were observed in CA1 pyramidal cells and interneurons. In 70% of cases the phase of spike coding appeared without any difference in the firing rate. Co-occurrence of firing rate and the phase of spike coding was observed 4 times more often than expected by random distribution. Our data suggest that stimulus-specific spike timing patterns, together with general accuracy of neuronal phase-locking, are important for hippocampus-mediated associations of sounds and place.

Keywords: phase of firing coding, theta, oscillation, hippocampus, auditory

The respiratory neurons impulse activity changes upon some hypothalamus structures stimulation in hypoxia

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The study has been conducted on white rats during hypoxia: 1. in normoxia (pO2 = 142 mm Hg), 2. on the "altitude" of 4-5 thousand meters (pO2 = 98-85 mm Hg), 3. on the "altitude" of 7.5-8 thousand meters (pO2 = 64-58 mm Hg), which was constructed in altitude chamber, 4. after the "descent", in conditions of normal atmospheric pressure. The reaction of respiratory neurons upon hypothalamus stimulation in normoxia, i.e. before the animal 'ascent', worked as a control for the experiments in the condition of acute hypoxia.

In normal conditions we recorded 133 RN, of which 72 were EN and 61-IN. All subgroups of EN and IN reacted upon mamillary nucleus (MM) stimulation with a domination of arousal: for example, the impulse activity of the 63.8% EN and 65.6% IN became more frequent.

At the altitude of 4-5 thousand meters the 51 (70.8%) of EN and 45 (73.7%) of IN remained active, of which 60.7% EN and 62.2% of IN neurons responded to the MM electro-stimulation to be high impulse activity. On such background, the effect of hypothalamus' MM stimulation was less expressed.

At the 7.5-8 thousand meters, the change in the impulse activity of respiratory neurons was expressed in the decrease of impulse discharge, and in several cases—in complete inhibition of their activity. Meanwhile, only 40 (55.5%) of the EN and 36 (59.0%) of the IN continued to remain active. Reaction of these neurons on hypothalamus' stimulation was more expressed and activity.

Keywords: hypothalamus, hypoxia, respiratory neurons

RNA interference of cerebellar Cav2.1 calcium channels generate stress induced ataxia in adult mice

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The neuronal Cav2.1 channels are mainly localized in presynaptic nerve terminals where their opening is linked to the rapid release of vesicles. Cav2.1 is particularly highly expressed in the Purkinje cells of the cerebellum and mutations in human cause several autosomal dominant disorders, including episodic ataxia type 2 (EA2). EA2 is characterized by variable symptoms like periodic attacks, gait ataxia and vertigo, and ataxic episodes in humans can be triggered by stress, exercise, caffeine and alcohol. In contrast, Cav2.1-/- mice as well as spontaneous mouse mutants with loss-of-function in the CACNA1A gene (tottering, leaner, etc.) show continuous and progressive ataxia questioning about the pertinence of these models to study EA2. In the present study, we have investigated the ability of RNAi strategy to suppress Cav2.1 expression in the cerebellum and to induce motor dysfunction. Following cerebellar injection of shRNA lentiviral based vector, Purkinje and granular neurons are the majority of transduced cells in new born mice whereas only Purkinje are transduced in adult. Indeed, Cav2.1-shRNA injected adult mice showed no basal behavioral impairment but stress-induced loss of motor coordination. These results demonstrate that Cav2.1-RNAi lentiviral strategy provides an effective model to dissect the subtle neuronal alterations that lead to EA2 symptoms in mice.

Keywords: Cav2.1 channel, episodic ataxia type 2, cerebellum

Early and late MRI changes in rat brain after prolonged seizures and nonspatial memory impairment

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Prolonged epileptic seizures are known to cause neuronal death and lead to brain damage. Lesions in various brain regions can result in memory and cognitive impairment. We studied early and late changes of brain structures after prolonged severe seizures on lithium-pilocarpine model of status epilepticus (SE) in rats. To induce SE, Wistar rats were treated with LiCl i.p., and pilocarpine i.p 24 hours after. Control animals received saline instead of pilocarpine. Seizures were observed and scored for 2 hours. MRI study of rat brain was performed 2, 7 and 30 days after SE. High-resolution T2 images and T2-maps were obtained, and total damaged area, hippocampal volume, and T2 coefficients in several brain structures were calculated. A week after the MRI study, animals were tested in an open field. To investigate the ability of the animals to habituate to the new environment, the test was performed three times with 24-hour intervals. After SE induced by pilocarpine, the increase of T2 signal was found in hippocampus and associated structures. The patterns of brain damage in rats after SE varied considerably. All rats after SE demonstrated high motor activity in an open field and did not habituate in the new environment that could be the evidence of long-term nonspatial memory deficit. Rats with large increase of T2 signal and considerable early changes found by MRI, tended to demonstrate higher activity in the open field in comparison with rats with less pronounced early MRI changes.

Keywords: status epilepticus, magnetic resonance imaging, habituation, open field test

Parvalbumin neurons and calretinin immunoreactive fibers degenerate in the subiculum after kainate-induced seizures in the rat

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The subiculum, the major output area of the hippocampus, is closely interconnected with the entorhinal cortex (EC) and other parahippocampal areas. In animal models of temporal lobe epilepsy (TLE) and in TLE patients it exerts increased network excitability and may crucially contribute to the propagation of limbic seizures.

Using immunohistochemistry and in-situ hybridization we now investigated neuropathological changes affecting parvalbumin (PV) and calretinin (CR) containing neurons in the subiculum and EC after kainic acid (KA)-induced status epilepticus.

We observed prominent losses in PV containing interneurons in the subiculum and EC. Degeneration of PV-positive neurons was associated with significant precipitation of PV-immunoreactive debris 24 hrs after KA-injection. In the subiculum the superficial part of the pyramidal cell layer was more severely affected than its deep part. In the EC, mainly the deep layers were affected. The decrease in number of PV-positive neurons in the subiculum correlated with the number of spontaneous seizures subsequently experienced by the rats. CR-positive fibers terminating in the molecular layer of the subiculum, in hippocampal sector CA1 and in the EC degenerated together with their presumed perikarya in the thalamic nucleus reuniens.

Notably, the loss in PV positive neurons in the subiculum equaled that in human TLE. It may result in marked impairment of feed-forward inhibition of the temporo-ammonic pathway and may significantly contribute to epileptogenesis. Similarly, the loss of CR-positive fiber tracts originating from the thalamic nucleus reuniens significantly contributes to the rearrangement of neuronal circuitries in the subiculum and EC during epileptogenesis.

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Keywords: epilepsy, subiculum, kainic acid, animal model, entorhinal cortex, neurodegeneration

Hypoxic preconditioning abolishes changes of CRH and vasopressin expression in hypothalamus triggered by inescapable stress in animal models of depression and anxiety

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A preconditioning by using repetitive mild hypoxia exposures is known to induce a tolerance of the brain neurons to injurious factors including severe hypoxia and various stresses. Previously, we demonstrated antidepressant and anxyolitic effects of hypoxic preconditioning in rodent models of depression and anxiety.

The aim of the present study was to reveal the neuroendocrine mechanisms of this phenomenon. Animal models have been applied, including the learned helplessness as a model of depression and stress-restress paradigm as a model of anxiety disorder (posttraumatic stress disorder, PTSD, in particular). Rats were exposed to three trials of mild hypobaric hypoxia in a hypobaric chamber prior to inescapable stress exposure in both models used. Our previous findings have shown that depressive-like and anxiety-like states in rats are accompanied by significant changes of CRH and vasopressin expression in the neurons of hypothalamic paraventricular nucleus, suggesting putative triggering role of CRH and vasopressin systems in depressive- and anxiety-like states onset. Hypoxic preconditioning prevented CRH and vasopressin expression modifications in hypothalamic neurons in parallel with normalization of disturbances in hypothalamic-pituitary-adrenal axis functioning. Present findings reveal CRH- and vasopressinergic mechanisms of antidepressant and anxiolytic effects of hypoxic preconditioning, and suggest hypoxic preconditioning as an effective non-pharmacologic tool for the prophylaxis of stress-related affective pathologies in humans.

Keywords: hypoxic preconditioning, animal models of depression and anxiety, hypothalamus, CRH, vasopressin

Ingrowth of sensory axons into end-to-side neurorrhaphy – a retrograde tracer study in rat

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After end-to-side nerve repair the donor nerve axons are able to grow into the recipient nerve stump. It is unclear, however, if these ingrown axons are collateral sprouts of uninjured donor nerve axons or regenerating axons of injured donor nerve axons.

The distal stump of transected peroneal nerve (recipient) was sutured to side of uninjured ipsilateral sural nerve (donor) in rat. At the time of coaptation (control group), 7 (group I), 28 (group II) or 84 days later (group III; n = 8 for each group), retrograde tracers 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) and Fluoro Gold (FG) were applied to recipient and donor nerves, respectively, just distally from the coaptation site. After 10 days of recovery, DRGs L4-L6 were harvested, cut on freezing microtome, and all single and double labeled neurons on DRGs sections were counted.

Double labeled neurons represented 50%±18%, 66%±17%, 28%±10% and 24%±10% (mean±SD) of all neurons labeled from the recipient nerve in control group (121±54), group I (206±118), group II (327±158) and group III (317±101), respectively. The percentage of double labeled neurons in control group or group I was statistically significantly different from group II or group III ($p < 0.05$). Differences in the numbers of all labeled neurons were statistically significant between control group and groups II or III ($p < 0.05$).

Our results suggest, that early after an end-to-side nerve repair both the collateral sprouts of uninjured and the regenerating axons of injured donor nerve sensory axons take about equal portions in reinnervation of the recipient nerve stump.

Keywords: end-to-side neurorrhaphy, sensory axons, peripheral nerve, rat

Neonatal exposure to organophosphorous substance chlormephos affect anxiety-like behaviour in adult mice, but does not permanently disrupt blood brain barrier

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Organophosphorous substances are used as pesticides and as nerve poisons. Acute effects of organophosphorous substances are well known, but much less is known about potential long term effects of low doses of such chemicals. Some studies have shown that organopshophoroous compunds could disrupt blood-brain barrier and thus affect brain function. To examine potential effects of exposure to low doses of organophosporhous compounds, female mice were treated with low doses of chlormephos during pregnancy and lactation. In offspring of treated mice that were exposed to chlormephos only through mothers until 3 weeks of age, anxiety like behaviour and brain microstructure were examined in adult life. Behavioral analyses using elevated plus maze revealed increased anxiety like behaviour in mice that were neonatally exposed to chlormephos. For transmission electron microscopy, pieces of mouse brain tissue were fixed in a 4% glutaraldehyde and postfixed with OsO4. Ultra-thin sections were examined by the transmission electron microscope with special emphasis on the blood-brain barrier. In brains from control and treated mice, brain capillaries displayed normal morphology: endothelial cells displayed flattened nucleus and cytoplasm. Neighbouring endothelial cells were joined by junctional complexes and laid on a continuous basement lamina. There was no damage or disruption of the basement membrane and/or endothelial cell cytoplasm. In conclusion, our study revealed that exposure to low doses of organophosphorous compound chlormephos could have long term effect on behaviour, although the mechanism of this behavioural effect is not yet known and likely does not involve disruption of blood brain barrier.

The expression of cathepsin X and gamma enolase in mouse models of Alzeimer's disease and neuroinflammation induced by lipopolysaccharide

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Cathepsin X is a lysosomal cysteine protease involved in inflammation induced neurodegeneration. It was found to be upregulated in glial cells of degenerating brain regions in transgenic mouse models of ALS and Alzheimer's disease. Recent studies on PC12 cells have revealed that cathepsin X is able to cleave the γ-enolase by eliminating the neurotrophic activity of this isozyme. Here we aimed to characterize the expression of cathepsin X and γ-enolase in the proximity of β-amyloid plaques of transgenic Tg2576 mice, model for Alzheimer's disease and after intrastriatal injection of lipopolysaccharide, model for neuroinflammation. By the use of in situ hybridization with emulsion autoradiography we observed upregulation of cathepsin X and γ-enolase mRNA associated with β-amyloid plaques. Immunofluorescent staining confirmed the upregulation of cathepsin X around and γ-enolase in β-amyloid plaques, but the co-localization of these two proteins was visible only in individual cells. We also demonstrated the lipopolysaccharide-induced upregulation of striatal cathepsin X protein. Additional experiments are needed to find out whether cathepsin X cleaves γ-enolase in these particular pathological conditions.

Keywords: cathepsin X, γ-enolase, β-amyloid plaques, lipopolysaccharide, neuroinflammation

Comparative study of the influence of the acute administration of drugs of abuse on 50 kHz ultrasonic vocalization in male rats

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Increasing evidence indicates that the emisison of ultrasonic vocalizations (USVs) may index the emotional state of rats, with the 50 kHz component of these vocalizations reflecting a "positive" state. A thorough characterization of the pharmacological stimuli capable of triggering the emission of 50 kHz USVs is however lacking. On these bases, this study examined the influence of some psychoactive drugs bearing to different pharmacological classes on the emission of 50 kHz USVs by male rats. Rats received one of the following drugs, in acute administration: methylenedyoxymethamphetamine (MDMA 5-15 mg/kg i.p), methylphenidate (2.5-10 mg/kg i.p.),morphine (1-7 mg/kg s.c.), nicotine (0.1-0.4 mg/kg s.c.). Additional group of animals received the acute administration of D-amphetamine (2 mg/kg i.p.), which is known to robustly stimulate the emission of 50 kHz USVs, by term of comparison. The results obtained show that methylphenidate stimulated the emission of 50 kHz by rats in a fashion comparable to that of D-amphetamine, whereas MDMA, morphine and nicotine influenced the emission of 50 kHz USVs only marginally.

This study shows that important differences exist in the ability of psychoactive drugs to stimulate the emission of 50 kHz USVs by rats. The present findings may further elucidate the neurobiology of 50 kHz USVs and may help understanding the relevance of 50 kHz USVs to drug-induced reward.

Aversive effects of ethanol. Ethanol-induced conditioned taste avoidance in male Wistar rats

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The present experiment aimed to discern effects of two different doses of ethanol (12.5% (v/v) and 2 g/kg; 15% (v/v) and 1.5 g/kg) as unconditioned stimulus (US) in an ethanol-induced Conditioned Taste Aversion (CTA) task. In the CTA paradigm used, solutions of ethanol and saline (conveniently balanced) were administered on alternate days immediately after the intake of a palatable taste cue as conditioned stimulus (CS) during the acquisition period, according to experimental protocol established. Rats avoid intake of CS when it was paired with both doses of ethanol. The dosage resulted in no significant difference in ethanol-induced CTA in both cases. Additionally, it was observed a comparable failure to develop extinction of the suppression CS intake when rats were received 7-minute access to palatable solution, which was used as CS during the learning period, and no injection of the US during 8 days of extinction period. Moreover, this decrement in CS intake was maintained even after a period of two weeks that rats were ad-libitum fed and well hydrated with an ad libitum fluid intake, in which rats were subjected to other experimental procedures. These results and previous finding, that were obtained in our laboratory using female wistar rats (in press), suggest that suppressive effects are due to aversive properties of ethanol. Furthermore, other studies will be developed to determine the contribution of stress to the modulation of these aversive properties of ethanol after an addiction acquisition to ethanol and the role of both factors in susceptibility to alcoholism.

Keywords: ethanol, conditioned taste aversion, aversive properties

YNFL'11

Young Neuroscientist Forum Ljubljana '11

Faculty of Medicine University of Ljubljana

Ljubljana, Slovenia, September 22, 2011

Young Neuroscientists Forum Ljubljana 2011

Abstracts

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Faculty of Medicine, Ljubljana, Slovenia
22 September 2011

YNFL-01 Thursday, September 22nd, 09:00 [Student presentations]

Analysis of compound action potentials elicited in an insulated vagus nerve of a pig with selective vagus nerve stimulation

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One of the main functions of the vagus nerve is to monitor and control the activity of internal organs and glands such as the heart, lung, stomach, bladder and pancreas. For this reason, the use of vagal nerve stimulation (VNS) for treating and controlling a variety of medical disorders has seen significant growth over the last several decades. However, the VNS used worldwide is non-selective stimulation of the left vagus nerves and non-selective stimulation of fibers innervating a targeting organ. The result is the frequent occurrence of undesirable side effects.

The first specific purpose of this research is to develop the model, methodology and setup for using the multi-electrode stimulating system in selective activation of medium B-fibers within a particular superficial region of the insulated left vagus nerve of a pig using steered current quasi-trapezoidal current stimulating pulses and technique of subsequent anodal block.

The second specific purpose was to obtain information about the different fiber types stimulated. For this purpose conduction speed of compound action potential (CAP) in selectively stimulated myelinated nerve fibers, when stimulating pulses are applied to preselected locations along the nerve, has been precisely measured using particular recording electrodes within the cuff. Afterwards, the components of the CAPs recorded from corresponding compartments of the nerve were analysed.

The results show that the cuff and improved model of anodal block enable both, selective stimulation fiber activation and recording of CAP in B-fibers within particular compartment of a functional segment within the left vagus nerve of a pig.

Keywords: left vagus nerve, nerve fiber, vagal nerve stimulation, multi-electrode nerve cuff, compound action potential, conduction velocity

YNFL-02 Thursday, 22 September, 09:00 [Student presentations]

In vitro studies of EPM1 mutants of human stefin B

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Mutations in the gene of stefin B are responsible for the primary defect underlying EPM1. EPM1 is a rare progressive myoclonic epilepsy with generalized tonic-clonic seizures and slow progressive mental deterioration. Mutants by majority cause lower expression or loss of protease inhibitory activity, which would implicate lack of protein's protease function as the most likely cause. However, the physiological function of stefin B is still under investigation. The lack of stefin B increases apoptosis and oxidative stress in affected neurons, implying it has a protective role in the brain. The protein is also over-expressed in status epilepticus and after seizures, which could potentially cause its aggregation. It was predicted that stefin B as well as some EPM1 mutants might aggregate in cells (upon over-expression) and thus gain in "toxic function". Some pathological mutants of stefin B observed in EPM1 patients have been prepared and their stability and aggregation studied. Here we report on some new EPM1 mutants of stefin B: G50E and Q71P. Studies of their solution structure showed that they are partially unfolded and form a molten globule. This was confirmed by ANS fluorescence, which showed a large amount of exposed hydrophobic surfaces. No denaturation studies could be performed due to their instability. Similarly to WT and G4R mutant and the R68X fragment, the two mutant proteins (G50E and Q71P) formed fibrils at acidic pH even though their low ThT fluorescence values indicated differently.

Keywords: stefin B, EPM1, fibirllation

Investigating the "Tip-of-the-tongue" phenomenon

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Aphasia is a communication disability, usually caused by stroke. Patients seem to have a generic idea about the target word even when they cannot recall it. Such word retrieval difficulty appears to be similar to the commonly experienced "Tip-of-the-tongue" phenomenon (TOT) "...the state of mind in which a person is unable to think of a word that he is certain he knows..." (Brown, 1991). To explain the genesis of this state, Levelt (1999) proposed a hierarchical, feed-forward model of the processes that underlie word production. There are three main components: a concept stratum (forming focus on the appropriate contextual information); a word form stratum (retrieving the target word's structure); and an articulation stratum (forming the motor plan or engram for the word's articulation). In this serial model, failure at the 'word-form' level means that no information can be passed on to the next stratum where the 'engram' or motor program is assembled for articulation. In a TOT state, this is where the fault in naming appears to occur.

We induced a TOT state in 10 elderly subjects by asking them to name pictures of famous people (TOT rate = 32%). Brain activity was measured while the participants performed the task using magnetoencephalography (MEG). We identified a slow-wave component that was present for correctly named faces but was not present for TOT faces. We will present a source localization analysis of these difference waves to identify the neural structures that encode the long-term store of peoples' names.

Keywords: face-name retrieval, TOT phenomenon, magnetoencephalography

Changes at GABA-A receptors induced by long-term zolpidem treatment in primary culture of rat cerebellar granulle neurons

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Gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the mammalian brain, fulfills most of its physiological actions via GABA-A receptors. GABA-A receptors possess binding sites for a variety of different drugs, including clinically relevant benzodiazepines, barbiturates, general anesthetics and neurosteroides. Occupancy of these receptors by different drugs leads to regulatory changes often affecting receptor expression and/or function.

The aim of this study was to further explore the mechanisms leading to adaptive changes in GABA-A receptors following their prolonged exposure to zolpidem, a positive allosteric modulator of GABA-A receptors. Imidazopyridine zolpidem is the most widely prescribed non-benzodiazepine hypnotic, with preferential, although not exclusive, binding for receptors containing alpha1 subunit. It was suggested that drugs with high selectivity for alpha1 containing receptors produce, upon repeated treatment, less tolerance and dependence than classical benzodiazepines.

As an extension of our previous work, we treated cerebellar neuronal cells isolated from 8-days old rats with 10 microM zolpidem during 48 h. The results demonstrate that prolonged treatment of these cells with zolpidem induced changes neither in GABA-A receptor number nor in expression of alpha1 subunit mRNA. On the other hand, long-term exposure of these cells to zolpidem produced the functional uncoupling between GABA and benzodiazepine binding sites on GABA-A receptor complex as evidenced by a decreased ability of GABA to stimulate [3H]flunitrazepam binding.

We can assume that chronic zolpidem treatment might also induce tolerance, if this mechanism is responsible for the development of tolerance following chronic administration of classic benzodiazepines.

Keywords: GABA-A receptors, cerebellar granulle neurons, zolpidem

The influence of nanosize titania on rat EEG power

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The aim of research was to estimate the potential hazard of nanosize titania to higher nervous activity of rats. Thirty male Wistar rats were divided into three groups (N=10). Each group received a piece of attractive food with addition of nanosize titania ("nano" group), microsize titania ("micro" group) or only attractive food ("control" group). Rats had been receiving investigated material on a daily basis in mornings for 7 days. EEG was recorded before and after experiment. Needle electrodes were placed subcutaneously on parietal lobes and nasal bone.

Six band power (delta (0.5–4 Hz), theta (4-8 Hz), alpha (8-14 Hz), beta (14-30 Hz), gamma1 (30-49 Hz), gamma2 (51-70 Hz) were calculated.

Repeated measures group x time ANOVAs indicated no effect of electrode sites but indicated effect of time of EEG acquisition ($F = 8.855$; $p < 0.01$) and group of rats ($F = 10.481$; $p < 0.001$), as well as time x group interaction ($F = 14.529$; $p < 0.001$).

Wilcoxon matched pair test showed no significant differences in "nano" and "control" rats before and after experiment. Relative amplitude of theta, gamma1 and gamma2 band had significantly increased and delta had decreased in the right hemisphere of "micro" rats. So microsize titania had caused EEG frequency shifts which indicate increased brain activity. Relative amplitude of alpha and beta bands had decreased in left hemisphere of "nano" rats compared to "control".

Despite both of nanosize and microsize titania are considered to be safe materials nowadays, they had caused EEG changes in test rats, which means this phenomena requires further studying.

Keywords: EEG, spectral analysis, nanosize TiO2

A simple mathematical model accounts for the reactive electrocortical burst-suppression behavior during anesthetic coma

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Burst-suppression (BS) electrocortical pattern, consisting of "bursts" of activity on a suppressed/isoelectric background, accompanies deep comatose states. When the "spontaneous" bursting behavior can be modulated by external stimuli, the BS pattern is characterized as "reactive" and it is considered a marker of good prognosis for coma recovery. Nevertheless, current methods employed to quantify the functional cortical network impairment during BS do not account for its reactivity.

We investigated the reactive bursting behavior in response to visual stimulation (super-bright flashes delivered at 0.5 Hz in 1 min epochs) during chloral hydrate anesthetic coma in Wistar rats.

We found that during visual stimulation most if not all bursts occurred 200-300 ms after the flashes following a stereotypical behavior: (1) after the onset of stimulation increasingly more flashes failed to trigger bursts and (2) after the offset of stimulation the recovery of "spontaneous" bursting was delayed. The magnitude of these effects increased with the depth of anesthesia as reflected by the rate of "spontaneous" bursting. This complex reactive behavior could be simulated using a simple mathematical model considering that each burst causes a cumulative increase in the threshold for burst generation (TBG), followed by an exponential recovery with a time constant (Tau) proportional to the depth of anesthesia.

Our model suggests that during BS stimuli of various strengths compete to overcome the TBG, which depends on both the anesthetic depth and previous bursting history. We propose Tau as a reliable biomarker for monitoring the extent of functional connectivity impairment in the comatose brain.

Keywords: burst-suppression, coma, anesthesia, rat, visual stimulation

Brainstem tauopathy with progressive bulbar paralysis – a case presentation and analysis

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Alzheimer disease is the most common cause of dementia, pathologically defined by the presence of senile plaques and neurofibrillary tangles in cortical and subcortical brain regions. The brainstem is less affected, without obvious clinical manifestations. We report the results of brain examination of a patient with progressive isolated bulbar paralysis, without cognitive impairment and memory disturbances, which revealed severe brainstem tau pathology and changes corresponding to stadium II–III Alzheimer disease according to Braak.

Immunohistochemistry with antibodies against hyperphosphorylated tau protein (AT8), 3R and 4R isoforms of tau protein, and amiloid β were applied to tissue sections from the hippocampus and the brainstem of our case and of nine demented patients with Alzheimer disease. Gallyas silver impregnation method was used to visualize fibrillary tau pathology. Photographs of chosen areas from the sections labeled with AT8 were taken and analyzed to determine the intensity and the total surface area of the AT8 immunoreactive signal.

The neurodegenerative changes were morphologically similar in all analyzed brains. Both tau isoforms were expressed. The brainstem of our case was significantly more affected by tau pathology, which increased caudally, in the opposite direction relative to the brainstem tau pathology in Alzheimer disease patients, which shows an unusual distribution of neurodegeneration otherwise typical of Alzheimer disease.

We conclude the patient with progressive bulbar paralysis and severe brainstem tau pathology had the bulbar type of Alzheimer disease. This illustrates new differential diagnostic possibilities for Alzheimer disease and for vocal cords paralysis of unknown etiology.

Keywords: Alzheimer disease, brainstem tauopathy, bulbar paralysis

Research for pathophysiology of early complications in acute spinal cord trauma

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Objective:

1. Search for pathophysiological peculiarity and dynamics of early cardiac complications in acute spinal cord trauma

2. Watch for opportunities of local therapic destruction, using radiofrequency ablation in encephalon.

Methods:

Experiments were done with anesthetized 20–26 kg pigs (n = 6).

Spinal cord was opened and mechanical incision made in three different levels: cervical (C), cervical – thoracic (C - T) and lower thoracic (T). ECG, PR, BP, SN function, AV permeability and refracterity, respond to n. vagus stimulation were monitored.

The cranium was opened and radiofrequency ablation made to brain cortex. Damages were registered with thermovisual camera.

Results:

Hypotension (C: after 6 hours and later; C – T: after 4 hours and later; T: after 4 hours and later)

Bradycardia (C: after 30 min and later; C – T: after 2 – 6 min and later; T: after 30 min and later)

SN function suppression (C: after 30 min and later; C – T: after 10 min and later; T: after 1 hour and later)

AV permeability loss (C: no changes; C – T: 34 ± 7.1%; T: 18 ± 4.9%)

AV refracterity (C: no changes; C – T: increased; T: increased)

Increased respond to n. vagus stimulation (C: 11.6 ± 0.14%; C – T: 37.7 ± 2.78%; T: 21.4 ± 6.12%)

Radiofrequency ablation with 2 – 3W and 5 – 10s application made total speckle destruction, while ablation of 10W and more power made large and uncontrolled nervous tissue destructions.

Keywords: spinal cord trauma, cardiac complications, radiofrequency ablation

Influence of Mozart's sonata K.448 on visual attention performance

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The Mozart effect is neurophysiological phenomena, which results in better performance of spatial-temporal tasks after 10 minutes listening of Mozart's Sonata K.448. Since the impact of Mozart's Sonata K.448 on spatial-temporal reasoning has been well studied, this effect remains uncertain among other cognitive capabilities. One such uncertainty refers to visual attention. Although previous studies have discovered increased activation in visual-attention-relevant neuroanatomical structures (e. g. left prefrontal cortex) during and after the listening of Mozart's Sonata, impact on behavioral performance of visual attention haven't been studied yet. The purpose of our work was to study the impact of Mozart's Sonata K.448 on behavioral performance in visual attention task. In addition, we also checked if such impact remains constant or it tends to decline during task. 51 students of psychology (19 men and 32 women) were randomly assigned in experimental or control group and performed two parallel versions of visual attention task. Participants in experimental group were instructed to listen Mozart's Sonata K.448 between both tasks, while participants in control group took 3 minutes of rest. Participants in both groups performed two parallel versions of visual attention task. The results have shown that participants in both groups performed better in second task, but progress was significantly higher in experimental group. Besides, decline of visual attention was significantly slower in experimental group. Present study has shown that spatial-temporal reasoning is not the only cognitive capability that could be affected, although exact mechanism of observed changes remains unclear.

Keywords: Mozart effect, cognitive processes, visual attention

Correlation between results of cognitive tasks performed with different emotions and EEG high frequency band

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Individual approach can provide more effective results of a research. One of possible ways is to understand how various emotional impacts can influence cognitive processes. Thus, the aim of this study was to find correlations between emotional impact and power and coherency of EEG in gamma band.

We used emotionally positive and negative pictures and words, which were presented simultaneously with memory tasks or thinking tasks. In memory tasks subjects were asked to recall answers to questions, which had been memorized before. In thinking tasks subjects read two definitions and tried to find synonym for both of them (homonyms-method). EEG was recorded from 19 sites by 10-20% system. There were 23 subjects. The rest of EEG activity was recorded with open eyes for 20 seconds before cognitive tasks. The power of EEG were calculated in gamma1 (30-40 Hz), gamma2 (40-49 Hz), gamma3 (51-70) and beta (14-30 Hz) frequency bands. Spearman correlations ($p \leq 0.05$) were used to find correlations between the power and number of the correct answers.

It was found out that the higher was the power in every frequency band the better were the results of thinking task with emotional impact of positive pictures as compared to neutral. At the same time, for neutral and negative pictures negative correlations were found in every frequency band. Thus, arousal by the negative pictures modulation was higher then optimum for thinking tasks. And the neutral pictures did not have any significant influence to the arousal in contrast to the positive.

This research was supported by RFH, grant № 11-36-00372a2.

Keywords: EEG power, emotions, cognitive processes

An online brain-machine interface using decoding of movement direction from the human electrocorticogram

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Brain-machine interfaces (BMIs) can be characterized by the approach used to translate brain signals into effector movements. Here we use a "direct motor" BMI approach where movements of an artificial effector (e.g. movement of an arm prosthesis to the right) are controlled by motor cortical signals that control the equivalent movements of the corresponding body part (e.g. arm movement to the right). This approach has been successfully applied in monkeys and humans by accurately extracting parameters of movements from the spiking activity of multiple single-units. We show that the same approach can be realized using brain activity measured directly at the surface of the human cortex (electrocorticogram, ECoG).

Five subjects suffering from intractable pharmaco-resistant epilepsy participated in the study after giving their informed consent. As a part of pre-surgical diagnosis all subjects had 8x8 ECoG grid implants over the hand/arm motor cortex. Subjects interacted with an experimental paradigm shown on a computer screen instructing subjects to move the hand contralateral to the side of the implantation. Subsequently, cursor on the screen was moved according to the movement direction decoded from the subjects' ECoG signals. Significant BMI control was achieved for 4 out of 5 subjects with correct directional decoding in 69%-86% of the trials (76% on average).

Our results demonstrate the principle feasibility of an online direct motor BMI using ECoG signals. Thus, for a direct motor BMI, ECoG might be used in conjunction or as an alternative to the intra-cortical neural signals, with possible advantages due to reduced invasiveness.

Keywords: BMI, BCI, ECoG

Morphological and quantitative analysis of neurons in lateral human hypothalamus and distribution of OX1R receptors

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The lateral hypothalamic area (LH) of human hypothalamus is the most prominent part of the lateral hypothalamic zone.

The aims of the present study are: (1) to detail the dendritic morphology of Golgi impregnated neurons from the LH and (2) to describe and quantify the dendritic branching features, in an attempt to identify a cytoarchitectonic model for hypothalamic neurons and (3) to describe the OX1R immunoreactivity of lateral hypothalamic neurons. The study is based on 7 hypothalamus' of young individuals who died accidently (Department of Forensic Medicine and Toxicology, Aristotle University of Thessaloniki). The impregnated neurons were analyzed with an image analysis software tool (ImageJ) for the following parameters: number of primary, secondary, tertiary and quartiary dendrites, length of each category of dendrites, total length of dendrites. Finally a Sholl analysis was performed.

Results:

1. The neuronal body protrude 2 or 3 primary dendrites (2-3) which are short in length (39.38±17.69μm).

2. The primary dendrites protrude 4 to 8 secondary dendrites (mean 5.56) which are rather long up to 175 μm (mean length 107.1) and with total length 580.91 ± 270 μm.

3. The total number of dendrites appears to be relatively uniform with mean number 14.16.

4. The Sholl analysis for neurons of Lateral Hypothalamic Nucleus demonstrates a rather uniform pattern of dendritic organization.

5. The large polyhedral neurons of lateral hypothalamic area are strongly immunopositive for OX1R and the receptors are located mainly in the cytoplasm of neurons.

Keywords: morphology, quantitative analysis, lateral hypothalamus, orexin receptors

Electroencephalographic and behavioural effects of intraperitoneal injection of grayanotoxin in adult Genetic Absence Epilepsy Rat from Strasbourg (GAERS)

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Grayanotoxin (GTX) is found in honey obtained from nectar and pollen of Rhododendron and Rhododendron like plants from Ericaceae family. Excessive amount of mad honey containing GTX cause dizziness, hypersalivation, vomitting, seizures and convulsions. Further, the cardiodepressive effects of GTX are mediated through vagal nerve stimulation at the periphery. Our aim was to investigate the changes in spike-and-wave discharge (SWD) and motor movements when mad honey extract containing GTX is applied intraperitoneally to GAERS. Adult male GAERS were implanted with bilateral cortical recording electrodes. After recording of baseline EEG (PowerLab 8S) flower honey extract was applied ip. EEG and behaviours were monitored for 3 hours (9:30–12:30 a.m.). After a 2-day wash out period the extract corresponding to 5 mg mad honey was injected. EEG and behavioral changes based on the Racine's seizure scale were evaluated. The administration of flower honey extract did not cause any change in EEG or behaviour. Before the mad honey injection mean SWD duration was 713.0 ± 34.2 seconds in 30-minute-EEG recording. After the injection mean SWD duration significantly decreased to 243.8±114.4 seconds in first 30 minutes ($p < 0.05$), 294.8±82.5 seconds in second 30 minutes and 556.6 ± 138.8 seconds in third 30 minutes. Latency to the first behavioral changes was 232.8 ± 97.4 seconds after the injection. Changes in behavioral activity reached maximum of stage 4 in the 30-60 minutes and disappeared 90 minutes after the injection. The administration of mad honey extract containing GTX causes a decrease in SWD activity in EEG and leads to accompanying behavioural changes in GAERS.

Keywords: grayanotoxin, mad honey, GAERS, seizure

Cognitive function in adults with type 1 diabetes mellitus

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Individuals with diabetes are approximately 1.5-fold more likely to experience cognitive decline than individuals without diabetes. The Stroop Neuropsychological Screening Test and Wisconsin Card Sorting Test (WCST) are widely used in clinical practice and research to examine selective attention, cognitive flexibility, processing speed, the ability to learn concepts, and used as a tool in the evaluation of executive functions.

The objective of our study was to investigate differences in cognitive function between patients with type 1 diabetes mellitus (T1DM) and healthy controls (C), and evaluate the association between cognitive dysfunction and glycemic control in patients.

37 T1DM and 37 C were screened for cognitive function with the Stroop test and the WCST. Depression was evaluated with the Hospital Anxiety and Depression Scale. Interview questionnaires surveyed the detailed anamnesis and medication.

The Stroop test showed that the common reaction time (RT) (DMRT: 932.17±118.67ms), the congruent RT (DMCRT: 825.89±94.26ms) and the incongruent RT (DMICRT: 946.81±126.25ms) of the T1DM group were significantly ($p < 0.01$) longer than the C group common RT (CRT: 705.81±96.16ms), congruent RT (CCRT: 646.86±79.87ms), and incongruent RT (CICRT: 766.95±124.61ms). A statistically significant association was observed between the HbA1C (7.81±1.75%) levels and the score on Stroop effect. In WCST the rate of correct answers were significantly ($p=0.008$) less in T1DM.

T1DM is associated with cognitive deficit, which can be related to many different regions of the brain. Our results indicate that T1DM show impaired executive functions and information-processing speed. Chronic poorly controlled carbohydrate metabolism might have a role in the development of selective attention deficit. Optimal diabetes care can improve the motor speed and attention.

Keywords: diabetes, cognitive function, Stroop task, WCST

Multisensory integration in primary and supplementary visual cortices of the mouse: an in vivo 2-photon-calcium imaging study

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We know a substantial amount about how neurons in the sensory areas of the brain are tuned to visual, auditory, and tactile stimuli. At the next processing level, these single modality representations are thought to be combined and grouped into multisensory representations. While a considerable amount of animal research focuses on multisensory integration in primates and cats, it remains unclear whether multisensory neurons exist in primary and supplementary visual cortical areas of the mouse and, assuming they do exist, whether they show spatial organization.

In vivo 2-photon-calcium imaging with multi-cell bolus loading of a calcium indicator (Oregon Green BAPTA 1-AM) will be used to locate and identify multisensory areas in the mouse visual cortices. The mouse will be presented with visual, auditory, and tactile stimuli that will occur in isolation, in pairs, and all together. Once multisensory areas will be identified, the functional micro-organization will also be examined. In addition, I will explore whether the neural computation underlying multisensory responses reflects super- or subadditivity.

Based on anatomical clustering of afferents in mouse visual areas it is predicted that auditory, visual, and somatosensory inputs form sensory hot-spots within primary and supplementary visual cortices. Furthermore, it is expected that multisensory neurons will exhibit both superadditivity and subadditivity of responses to multimodal stimuli.

Keywords: multimodal, 2-photon-calcium imaging, mouse, visual cortex

Neurofeedback training of the upper alpha frequency band in EEG improves cognitive performance

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In this work, the individually determined upper alpha frequency band in EEG (electroencephalogram) was investigated as a neurofeedback parameter. Trainability, regulation independent of other frequency bands and effects on cognitive performance were used as validation parameters.

Fourteen subjects were trained on five sessions within one week by means of feedback dependent on the current upper alpha amplitude. On the first and fifth session, cognitive ability was tested by a mental rotation test.

As a result, eleven of the fourteen subjects showed significant training success, revealing an enhancement of upper alpha EEG activity that was absent in a control group which did not receive feedback. Additionally, upper alpha was increased independently of other frequency bands. At the same time, enhancement of cognitive performance was larger for the the neurofeedback subjects than for the control group, indicating behavioral relevance of neurofeedback. Only in the neurofeedback group, improved cognitive performance went along with an increased upper alpha amplitude.

Thus, this work revealed the upper alpha band as a promising parameter for neurofeedback and confirmed a hypothetical relation between upper alpha and cognitive performance.

Keywords: neurofeedback, EEG, upper alpha, trainability, cognitive performance, mental rotation

Phase of firing coding of sounds in the hippocampus

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Encoding of sensory events in hippocampus is crucial for episodic memory. Odors, sounds and textures cause firing rate changes in hippocampal neurons, which are typically measured on the order of hundreds of milliseconds. Yet, fine temporal precision of neuronal activity is crucial for associative plasticity. How sensory events are encoded in the temporal patterns of spikes was the question of our study.

Four stimuli two-alternative forced choice discrimination task was designed to disentangle neural activity related to sounds and place. Sound-specific changes in the preferred phase of firing and alteration of phase-locking strength were observed in CA1 pyramidal cells and interneurons. In 70% of cases the phase of spike coding appeared without any difference in the firing rate. Co-occurrence of firing rate and the phase of spike coding was observed 4 times more often than expected by random distribution. Our data suggest that stimulus-specific spike timing patterns, together with general accuracy of neuronal phase-locking, are important for hippocampus-mediated associations of sounds and place.

Keywords: phase of firing coding, theta, oscillation, hippocampus, auditory

Anatomical, neurochemical and functional consequences of selective cholinergic lesioning combined with local infusion of pre-aggregated amyloid peptide

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Cholinergic loss, senile plaques and tau hyperphosphorylation are important hallmarks of Alzheimer's Disease (AD), however it is yet not known whether and to which extent are interactions between these features required to produce the complex spectrum of pathophysiological changes detected in AD patients. In the present study, the possible existence of functional relationships between the various hallmarks, and their role in producing cognitive impairments have been addressed by combining selective lesioning of the basal forebrain cholinergic neurons with the intrahippocampal injection of pre-aggregated beta (25-35) amyloid peptide, the latter giving rise to local accumulation of oligomers and protofibrils. To this aim, four to five weeks post-surgery, the animals were subjected to sequential testing in several spatial learning and memory maze tasks designed to evaluate reference and working memory abilities, followed by post-mortem morphological and neurochemical assessments. The results show dramatic deficits in both reference and working memory, associated to the occurrence of amyloid aggregates in the neocortex and hippocampus, as well as widespread cholinergic depletions and marked regional increases of APP protein levels, which were more pronounced in the animals subjected to double, but not to either single treatment. The results suggest that the presumed neurotoxicity of the various forms of amyloid requires association with disturbances in monoaminergic (e.g., cholinergic) neurotransmission for inducing cognitive impairments, thus supporting the hypothesis of important functional interactions between these events. Data will also be presented substantiating, in impaired double-lesioned animals, the functional recovery promoted by novel compounds with pro-cognitive and neuroprotective actions.

Keywords: Alzheimer's disease, immunotoxin, amyloid, cognition

Noradrenergic contribution to spatial learning and memory: effects of selective lesion and tissue transplants

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Severe loss of noradrenergic neurons in the Locus Coeruleus/SubCoeruleus (LC/SubC) complex and of fiber terminals in the neocortical and hippocampal target regions is known to occur in Alzheimer's disease (AD), however, the exact role played by such transmitter system in the pathophysiology of AD is still unclear. In the present study, the noradrenergic contribution to cognitive functions was investigated following bilateral stereotaxic infusion of the selective noradrenergic immunotoxin anti-dopamine beta-hydroxylase (anti-DBH)-saporin, into the LC/SubC complex of the adult rat. Four to five weeks post-surgery, the animals underwent behavioural tests, administered in sequence, to evaluate reference and working memory abilities, followed by semi-quantitative immunohistochemistry to assess the extent and selectivity of the lesion. When tested in the Morris Water Maze (MWM) task, lesioned animals exhibited only very mild or no deficits in reference memory compared to Normal. By contrast, working memory abilities (assessed by the Radial Arm Water Maze, RAWM task) in these animals were seen significantly impaired, both in terms of latency and errors. Post-mortem morphological analises, carried out on fixed brain tissue, confirmed a massive loss of immunoreactive neurons in the LC/SubC complex, associated to a virtually complete denervation of target areas in the neocortex and hippocampus. The results indicate the existence of a dissociation in the functional effects of the selective lesion, which suggests a role for ascending regulatory noradrenergic afferents in more complex aspects of cognitive performance (i.e. working memory). Data will also be presented concerning the functional effects of implanted progenitors in ameliorating cognitive performance.

Keywords: Alzheimer's disease, noradrenaline, selective lesion, cognition, cell transplantation

Neural stem cells-enriched tubulization improves anatomical and functional restoration of severed rat sciatic nerve

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Tubulization of the severed peripheral nerve has recently emerged as a promising strategy to promote nerve reconstruction and functional restoration of the affected limb. Interestingly, neurotubules can be loaded with growth-promoting factors or cells, which may speed-up and/or optimize the final outcome, however, only relatively scant evidence is available. In the present study, we have investigated this possibility after the formation of a 5-mm gap and tubulization of the rat sciatic nerve with a cell-compatible, biodegradable PLCL (poly DL-lactide-ε-caprolactone, Neurolac®) copolyestere tube filled with either cultured human neural progenitors or with their conditioned medium. The in vivo analyses included also groups of animals subjected to direct suturing of the transected sciatic nerve, animals with a gap and no implant, and animals implanted with unloaded PLCL tube. Starting from one week post-surgery the animals underwent weekly evaluation of the lesion and treatment effects, by analysing indexes of sensory-motor function of the lesioned limb, as compared to the intact contralateral side. At the end of the experimental period (5 months) histochemical and retrograde fluorescent tract-tracing procedures were carried out to assess the anatomical and functional integrity of the nerve. The results showed a better functional recovery in the animals with no gap and direct suturing and in those implanted with the cell- or medium-loaded tube compared to the other groups. Thus, tubulization associated with local supply of growth-promoting factors may represent a viable strategy for functional nerve reconstruction, but further detailed analyses are needed before traslating it into clinical use.

Keywords: sciatic nerve transection, tubulization, neural stem cell, implantation, recovery

An immunocytochemical tracer study of nigral dopamine neurons for the simultaneous double visualisation of tyrosine hydroxylase and fluorogold in light microscopy to investigate neuroprotection in a rat's model of Parkinson's disease following deep brain stimulation of the subthalamic nucleus

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Continuous deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an established neuromodulative therapy in the treatment of Parkinson disease (PD). Preclinical research in animal models of PD showed a rescue of nigral dopamine (DA) neurons after chronic unilateral or bilateral STN-DBS, respectively. However, from these studies one cannot conclude that neuroprotection comprises the total population of nigral neurons.

We aimed to establish a double staining immunocytochemistry protocol for an endogenous marker (tyrosine hydroxylase) and an exogenous marker (fluorogold) of nigral DA neurons which will enable more accurate investigation of the pattern of nigral neuroprotection following STN-DBS in the 6-OHDA rat model of PD.

In order to differentiate DA-ergic neurons from the total number of nigral neurons we used the retrograde tracer FG to label these cells prior to the 6-OHDA lesion. FG was applied bilaterally at two injection sites of the rat striatum followed by unilateral striatal 6-OHDA lesion two weeks later. In the same surgical session a customized microstimulation system for rats was implanted inserting a monopolar electrode in the STN ipsilateral to the 6-OHDA lesion. STN-DBS was initiated five days after the application of the neurotoxin and maintained for two weeks. After sacrifice, nigral serial sections are prepared for simultaneous immuncytochemical detection of TH and FG using a peroxidise-anti-peroxidase and immunogold reaction, respectively.

This newly established immunocytochemistry protocol may allow us to distinguish silent from active and rescued DA-ergic neurons. This would bring more insight into the rescue mechanism of nigral dopaminergic neurons after STN-DBS.

Keywords: deep brain stimulation, nigral neuroprotection, double staining immunocytochemistry

Activity of SKa-31 against seizure-like events in rat organotypic hippocampal slice cultures

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sK-channel plays role in regulating neuronal excitability, thus it might be of interest to suppress seizure in epileptic patients. Based on role of sK channel we aimed to test enhancer of sK-channel in rat organotypic hippocampal slice cultures (OHSCs) against seizure-like events (SLEs) induced by 4-amino-pyridine or Mg+2 free ACSF. Slice culture were prepared from p5-p7 rat pups [1] and incubated with maintained condition i.e. 5% CO_2, 36.5°C. Cultures were fed every alternate day, whereas 7-14 days old cultures were used for electrophysiological recordings using interface setup.

SKa-31 (sK-channel enhancer) at dose of 150µM completely suppressed seizure-like events, standard anti-epileptic drugs sodium valporate (2mM) and carbamazepine (100µM) were also blocked SLEs induced by 4-AP. All drugs were unable to block status or late recurrent discharges induced by Mg-Free ACSF. Data suggest sK channel enhancer has potential to suppress acute seizures but not pharmacoresistant epileptic seizures.

Reference:

Kann O, Schuchmann S, Buchheim K, Heinemann U (2003) Coupling of neuronal activity and mitochondrial metabolism as revealed by NAD(P)H fluorescence signals in organotypic hippocampal slice cultures of the rat. Neuroscience 119:87–100.

Keywords: hippocampus, epilepsy, sK channel

EMG, SFEMG & US 2011

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Abstracts

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Cankarjev dom, Ljubljana, Slovenia
23—25 September 2011

Keynote Lecture

Ultrasonography of peripheral nerve & muscle in EMG lab

Luca Padua

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Ultrasound (US) is being used more and more frequently to evaluate the peripheral nervous system, thus influencing the diagnosis and clinical care of the symptomatic patient in a variety of clinical settings, such as inherited disorders, entrapment syndromes, traumas and tumors.

However, there is no real study about its possible significant role in routine practice. Recently we aimed to assess the contribution of US as a routine tool in a neurophysiological laboratory.

In our lab 130 patients were clinically, neurophysiologically and sonographically assessed in the same session by the same neurologist/neurophysiologist. Results showed a dramatic contribution in diagnosing but even more in providing crucial information for a better treatment.

US complements neurophysiological assessment even in routine practice, and this confirms the increasing interest in US for a multidimensional evaluation of peripheral nerve system diseases.

Recently, diagnostic imaging approach has dramatically improved its potential following the rate of computer technology evolution. If neurophysiologists are dealing with imaging as a mean to give a morphologic correlation to their functional parameters, imaging is also evolving toward new "functional" objectives.

After all, who is more accustomed to dealing with a "compound" response than neurophysiologists?

Theoretical principles of ultrasonographic diagnostics

Rok Hren

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The usage of ultrasound is widespread among the living organisms and is based on the concept of reflection of sound waves on surfaces between regions with different physical properties. To a large extent, the very same »techniques« that are used, e.g., by the bats to localize their prey or whales to communicate, humans employ to visualize and examine different parts of the human body. In fact, due to its obvious advantages (being simple, fast, and not utilizing ionizing radiation), the application of ultrasound has become one of the most often used diagnostic methodologies in medicine.

In diagnostic ultrasonography, we measure the time between emitted and received high frequency sound pulse. Since different organs have different acusto-mechanical properties, the portion of ultrasound wave is transmitted through the boundary surface, while the portion is reflected; from these reflections (echoes), we can construct the image. However, while the concept of ultrasonography is simple, there are some obvious technological challenges; for example, due to absorption of ultrasound waves in biological tissues, reflected signals are substantially attenuated, which needs to be taken into account when assembling the image.

In this overview, we will describe fundamental principles of ultrasonography, present how the image is formed (and the variables which may affect it), and discuss past, present, and future technological challenges in implementation of this invaluable and fundamental diagnostic modality.

Keywords: ultrasound, diagnosis, technological application

Friday, 23 September, 10:20

Ultrasound anatomy and examination techniques in common compression neuropathies

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One of the major advantages of sonography compared to other modalities for imaging of the soft tissues such as MRI and CT is its ability to acquire images in virtually every orientation along the course of a peripheral nerve. A well founded knowledge of regional anatomy and topography is important for the sonographic assessment. In the last few years sonography has developed into an extremely valuable tool as an adjunct to neurological investigations. Using high resolution ultrasound (HRUS) and especially high frequent transducers (> 12 MHz), features such as nerve epineurium and inner fascicular structure can be visualized and pathological changes such as nerve edema, echotexture abnormalities or nerve compression can be demonstrated.

Generally speaking neuropathy caused by extrinsic compression may occur anywhere in the body and affect a variety of peripheral nerves. Special anatomic conditions, however, may result in an increased risk for the development of so-called entrapment neuropathies at certain locations. Carpal tunnel syndrome (CTS) is the most common entrapment syndrome of the upper limb followed by the sulcus nervi ulnaris syndrome (SNU). Sonography is a valuable tool for the characterization of such compression neuropathies as well as the exclusion of secondary causes such as ganglion, tumors, inflammation, accessory muscles and functional features. Supinator syndrome (compression of the deep branch of the radial nerve) and tarsal tunnel syndrome are other entrapment neuropathies which can easily be detected by means of HRUS.

Friday, 23 September, 11:00

Pathological changes in peripheral nerves

Roman Bošnjak

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Microsurgeons have a specific possibility to evaluate focal and diffuse peripheral nerve lesions by direct vision (under microscope magnification), by palpation and by microsurgical dissection. Inspection includes evaluation of surface, shape, color, course and relation of the nerve trunk to the surrounding tissues. The spindle shaped expansion of the nerve with normal epineurium is usually a feature of benign intraneural tumor or traumatic neuroma. The nerve may also be found locally athrophic and transparent (e.g. in long lasting entrapment or compression) or thinner or thicker in general (in neuropathies). Palpation can reveal fibrotic segment of the nerve, usually far away from injury site (traction injury), fibrosis or gap at the exact injury site or neuroma-in-continuity or terminal neuroma. Discoloration of the nerve is revealed by loss of shiny and pearly white color, which is substituted by paleness and livid color, the capillary net is scarce. The nerve is easily dissected out from surrounding tissue in entrapments (neurolysis), but with difficulty from the scar or when infiltrated. Funiculolysis provides further information on the internal structure of the nerve. In complete lesions, the cross-section of the stump gives information on the vitality of the nerve stump and the resection of the nerve into healthy part.

These main physical characteristics of the surgically exposed nerve appear in specific patterns, that help microsurgeon to differentiate between traumatic and non-traumatic, tumorous and non-tumorous, benign and malignant peripheral nerve lesions early in the course of intraoperative exploration and to decide for the best microsurgical treatment. Exploration using intraoperative nerve action potential recording and nerve biopsy to guide the choice of surgical procedure are often useful. Intraoperative exams of some illustrative peripheral nerve lesions will be presented.

Friday, 23 September, 11:30

Ultrasound guided neuromuscular interventions: nerve-instillations and biopsies

Alexander Loizides

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Interventional procedures on peripheral nerves refer to a wider and heterogeneous rang of invasive procedures. In the past, regional anesthetic blocks were performed using palpable anatomical landmarks, and by eliciting paresthesia and/or electrical nerve stimulation the anesthesiologist was able to ascertain the proximity of needle to the nerve. These blind techniques brought into question other factors including onset, quality, safety and patient comfort. Regional anesthesia and biopsy of neural lesions can be done safely and successfully using ultrasound (US) as an imaging guidance to avoid relevant adjacent structures such as vessels and tendons. The primary advantage of US is the direct visualization of peripheral nerves with high spatial and contrast resolution, the portability that allows the examination at the bedside and the wide availability and low cost of this technique. In addition, sonography allows for the detection of anatomical variants, which is one of the main reasons that conventionally administered blocks might fail. US-guided interventional procedures are performed in real time, rapidly, efficiently and enable visualization of the needle tip continually during advancement and assuring that the needle is placed precisely in the desired location.

US-guided biopsy of musculoskeletal and neural lesions, US-guided therapy with local corticosteroids (e.g. carpal tunnel syndrome, Morton's neuroma, Meralgia paresthetica) and US-guided phenol injection in painful stump neuromas are only a few of the interventional procedures which are performed presently using US guidance.

Friday, 23 September, 12:00

Setting-up of ultrasonographic activity – our experience

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Although electrodiagnostic (EDx) studies are of paramount importance in evaluation of patients with suspected neuromuscular conditions they are unable to provide a definitive etiological diagnosis, and to differentiate neurotmesis from axonotmesis. Therefore we observed with interest development of ultrasonographic (US) methods for examination of the peripheral nerves and muscles.

Since the beginning of the year 2010 till the end of May 2011 we examined 237 different nerves in 193 patients (bilateral median or ulnar nerve exam were counted as a single nerve). The most common conditions evaluated were suspected median nerve entrapment neuropathy at the wrist and the ulnar neuropathy at the elbow. For visualization of the peripheral nerves we used standard US equipment (ProSound Alpha 10, Aloka Inc., Tokyo, Japan), and a linear 7-12 MHz probe.

We found pathologic US findings in 41% of patients with normal EDx findings in spite of clinically typical carpal tunnel syndrome. Ulnar neuropathies at the elbow were more common on the left (usually non-dominant hand) than on the right (53 vs. 22, respectively; $p < 0.05$). However, the percentage of the thickened ulnar nerves was significantly higher at the right compared to the left elbow (64% vs. 40%, respectively; $p < 0.05$). We also found the ulnar nerve swelling at the elbow associated with EDx more severe lesions (sensitivity: 30% in demyelination vs. 66% in axonal lesion; $p < 0.05$).

We found US to increase sensitivity of EDx, particularly in suspected carpal tunnel syndrome, improve localization of nerve lesion particularly in the ulnar neuropathy at the elbow. We also diagnosed entities we have never diagnosed before (e.g., tibial neuropathies in the thigh). We expect to improve our recommendations for surgical explorations of the nerves, plan to perform US guided nerve injections, and apply US methods also to muscle disorders.

Friday, 23 September, 14:00

Ultrasound guided pain management in the cervical and lumbar spine

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Cervical and lower back pain are very common conditions - in fact most individuals will experience neck and/or low back pain at least once in their life, and with increasing age a greater number of patients with such symptoms are seen by physicians. Aside from physical therapy and other rehabilitative methods, injection therapy (steroids, local anesthetics) targeted to the facet joints or to the nerve roots is well established in the treatment of cervical and lumbar pain. Patients with cervical and lower back pain can make a satisfactory recovery when treated with pararadicular corticosteroid injections or a combination of corticosteroids and local anesthetics for facet joints.

Imaging guided pararadicular and facet joint injections in the cervical and lumbar spine are to date mainly performed under CT or fluoroscopic guidance. US is already used successfully to guide a variety of instillation procedures in different anatomical regions showing many benefits: direct visualization of the target of interest, real-time needle guidance, visualization of the spread of local anesthetics and thus minimal risk of complications, a potential for dose reduction of local therapeutics, shortening of procedure time and the lacking of exposure to ionizing radiation. We present alternative, simple and easy to learn US-guided techniques for injection therapies in the cervical and lumbar spine.

Friday, 23 September, 14:30

Ultrasound imaging of iatrogenic and other nerve lesions

Michaela Plaikner

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Peripheral nerve lesions primary due to trauma and secondary to various surgical procedures are not uncommon. Besides the medical impact of these lesions they constitute a major social problem, as recovery is often incomplete which may result in a long lasting disability. Generally a nerve can be injured directly during blunt or sharp trauma and indirectly by laceration or misplaced fracture fragments which pierce or jam a nerve or by traction over bone or bone fragments. Additionally nerve lesions may occur primarily during surgical procedures (dissection of the nerve, traction, insertion of retractors, thermal injuries) or secondarily due to excessive scar formation or compression by hematomas. Furthermore orthopedic procedures may potentially harm peripheral nerves (direct damage by a surgical implant).

Detailed information about the type and extend of neural damage is crucial for the selection of adequate treatment. In the last few years sonography has developed into an extremely valuable tool as an adjunct to neurological investigations. Using high resolution ultrasound (HRUS) and especially high frequent transducers (> 12 MHz), neural damages of peripheral nerves can clearly be visualized.

Saturday, 24 September, 09:15

Introduction to single fiber EMG

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The technique of SFEMG uses a concentric needle electrode with a small recording surface to identify action potentials (APs) from individual muscle fibers. This allows measurement of the neuromuscular jitter - the variability in the time when APs are generated at the endplate - and the fiber density (FD), which represents the focal concentration of muscle fibers within the motor unit territory.

Fiber density is measured by positioning the electrode to record single voluntarily activated APs with maximal amplitude, then counting the number of time-locked APs. The FD is calculated as the average number of time-locked APs (including the triggering potential) in 20 different sites throughout a tested muscle. Increased FD is a sensitive measure of reinnervation and is also seen in some myopathic conditions.

Jitter results from fluctuations in the time it takes for endplate potentials (EPPs) to reach the threshold for AP generation and can be measured during voluntary activation or during axonal stimulation. In the former, the electrode is inserted into the tested muscle during slight activation and positioned to record APs from two or more muscle fibers that are time-locked, indicating that they belong to the same motor unit. The variability in the intervals between two APs during an epoch of time represents the jitter at the two endplates. During axonal stimulation, jitter is measured between the stimulus and APs from single muscle fibers and thus represents the variability at only one endplate.

Jitter becomes increased whenever the ratio between the AP threshold and the EPP becomes increased. When neuromuscular transmission is sufficiently impaired, nerve impulses fail to elicit muscle APs and impulse blocking is seen. Jitter varies among different endplates in a muscle and from muscle to muscle.

Jitter is calculated as the mean value of consecutive differences of intervals (MCD) from the following formula:

$$MCD = (| IPI_1 - IPI_2 | + | IPI_2 - IPI_3 | + .. + | IPI_{n-1} - IPI_n |) / (n-1)$$

where IPIi is the interpotential interval (or, during axonal stimulation, the stimulus-response interval).

The IPI may be influenced by the preceding interdischarge interval (IDI); when the IDI is not constant, as during voluntary activation, this may increase the MCD due to variability in the velocity of AP propagation along the muscle fibers. This effect can be minimized by sorting the IPIs according to the length of the preceding IDI and then calculating the mean of the consecutive IPI differences in the new sequence - the mean sorted-data difference (MSD).

Saturday, 24 September, 09:45

Advanced neurography & late responses: update

Erik Stålberg

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When a motor nerve is stimulated, late responses may be seen after the evoked CMAP, some of which carry important information regarding pathology. Generator mechnisms and clinical use will be discussed.

The most commonly observe late response is the so called F-waves. They typically vary in shape since they represent activity from individual axons in which the distal stimulation has evoked an antidromically volley that occasionally returns along the axon in orthodromic direction to the muscle. These F-waves represent single or summated motor unit potentials recorded with surface electrodes. Their latency is a good estimate of the conduction velocity along the entire nerve. This is often even more sensitive to general slowing than conventional nerve conduction parameters. Tibial and ulnar nerves show most F-waves, median nerve less and peroneal nerve usually very few. In situation with nerve conduction block, no F-waves appear.

Another response is the so called A-wave, seen as waves with constant latency and shape at repeated stimulation. These are usually generated along an abnormal nerve when an antidromic volley is passing a hyperexcitable site. The A-waves can be seen in normal normal tibial nerve, but indicate pathology when seen in other nerves. Their latency may be short, just after the CMAP, usually longer and sometimes longer than the F-wave latency.

A similar constant response is the CMAP satellites. These reflect distally abnormally slow conduction axons and can be seen both in demyelinating and axonal neuropathies. They can be differentiated from the A-waves.

Other late responses are reflexes or various kinds not discussed here.

Saturday, 24 September, 10:15

Stimulation SFEMG: the need for good technique

Jože Trontelj

Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Slovenia

Microstimulation of motor axons is conveniently used with single fiber EMG (SFEMG) for studies of neuromuscular transmission (NMT). Selective activation of small numbers of muscle fibers is achieved by using near nerve or intramuscular needle stimulation with electrical pulses of low amplitude (< 1 to < 5 mA) and short duration (10-50 μs). Single fiber action potentials (SFAPs) are recorded with a SFEMG electrode or, at least in the facial muscles, with a standard concentric needle electrode. The jitter is defined as variation of latencies of consecutive responses, i.e. time measurement is made between the stimulus and a SFAP (rather than between two SFAPs as in the method in voluntarily activated muscle). This means that only a single NMJ is being assessed at a time and pairs or multiple SFAPs from the same motor unit are not required.

The technique has some attractive advantages over the standard jitter measurement in the voluntarily contracting muscle: (1) It offers perfect control of discharge rates. Low stimulation rates permit studying very sick neuromuscular junctions (NMJs), and high rates can be used to demonstrate presynaptic abnormality. (2) Rhythmic stimulation removes interdischarge interval dependent changes in muscle conduction velocity, improving the accuracy of results. (3) The stimulation technique does not require patient co-operation and can be used in little children, severely weak muscles, and experimental animals. (4) Often it is faster to perform, so larger number of NMJs can be evaluated.

There are however some pitfalls, which have to be carefully avoided. (1) Inadvertent threshold stimulation may result in false blocking and erroneously large jitter values. Each SFAP considered for measurement must be stimulated well above the threshold. Multiple SFAPs activated in each trace can be used for simultaneous measurement; however as they may belong to different axons, adequacy of stimulus strength must be checked for each of them. (2) Direct muscle fiber stimulation bypasses the NMJs and produces 'low' jitter. (3) In case of composite spikes, superimposition of two or several SFAPs tends to average-out the jitter of individual components, producing false low values. (4) Unrecognized axon reflexes may be misinterpreted as blocking SFAPs. When latency jumps are small they may be overlooked unless the sequential histogram is observed. (5) F-responses of the measured SFAPs produce saw-tooth like deformities in the sequential histogram; these may also increase the computed jitter. (6) Bimodal jitter is a rare source of erroneously high readings; it is not difficult to recognize and exclude.

A sample of 30-60 NMJs is usually collected per study, each represented with 50-100 responses. Up to 2 values outside the upper normal limit, with or without blocking, are accepted as normal.

Saturday, 24 September, 11:00

Keynote Lecture

The contribution of electromyography to the understanding of changes in myopathic muscle

Erik Stålberg

Department of Clinical Neurophysiology, Uppsala University, Uppsala, Sweden

The place for neurophysiological methods, particularly EMG, in the diagnosis and in understanding of myopathies is in focus of this presentation. EMG gives another type of information than that obtained with histochemical methods, imaging techniques and genetics. The functional aspect of nerve and muscle is the hallmark of neurophysiology.

In many muscle disorders, not only in myotonias, membrane hyperexcitability is present causing various patterns. With muscle at rest, spontaneous activity such as fibrillation potentials, complex repetitive discharges, and myotonic runs are seen in various combinations in different types of myopathies.

Changes in the EMG pattern at voluntary activation are related to abnormal fiber size, number of fibers, type grouping and fiber loss. These parameters can be quantitated and are therefore helpful in diagnosis and in follow up studies. These changes will be discussed using a simulation model.

In addition to conventional EMG methods, other techniques have helped to describe some of the changes in myopathy. Macro EMG gives the total electrical size of individual motor units, Scanning EMG gives a 2-dimentional picture of the motor unit.Valuable information obtained with methods such as multiarray recordings and intramuscular electrical stimulation will also be mentioned.

In general, the neurophysiological investigation gives quick information about functional parameters and is a useful tool for the first general description of type of muscle dysfunction. Combining results from EMG with findings from other methods may finally lead to a full description of the condition.

Saturday, 24 September, 11:30

Stimulated single fiber EMG at different frequencies in human botulism

José M. Fernández

Department of Clinical Neurophysiology, Complexo Hospitalario Universitario de Vigo (Xeral-Cies), Vigo, Spain

The low safety factor of neuromuscular transmission typical of presynaptic disorders (e.g. Lambert-Eaton myasthenic syndrome) when studied with single fiber EMG is known to improve by increasing the voluntary activation frequency or by high frequency (> 10 Hz) microstimulation resulting in lower jitter values and less frequent blocking. However, stimulated single fiber EMG has not been systematically used to test the function of end-plates in clinical botulism.

Five patients aged 30 to 54 (mean 43) with typical acute clinical botulism were studied using single fiber EMG with axonal microstimulation and the conventional methods. Stimulated SFEMG studies were performed in the frontalis muscles in all of them by applying rectangular pulses of 0.04 ms at frequencies ranging from 1-3 to 20 Hz with a monopolar needle electrode recording with a single fiber EMG electrode. We successfully collected sufficient data from the whole protocol in 28 end-plates.

Most end-plates showed high jitter values and intermittent blocking at lower rates of stimulation (1-3Hz) that improve after increasing stimulation frequencies (7-10Hz). At higher rates (20 Hz or more) there was a further improvement and only two end-plates showed deterioration with higher jitter values and blocking.

Most end-plates have a typical presynaptic disturbance. However, a small proportion of end-plates (about 10%) show a postsynaptic-like deterioration at higher frequencies of stimulation with increasing jitter and blocking.

Keywords: botulism, single fiber EMG

Saturday, 24 September, 12:00

Computer program for the electrodiagnostic evaluation of patients with suspected carpal tunnel syndrome

Simon Podnar

Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Slovenia

Median nerve entrapment at the wrist is the most common neuropathy, presenting clinically as "carpal tunnel syndrome" (CTS). Nerve conduction studies (NCS) are very useful to confirm CTS. The aim of the study was to analyze our first one-year experience with a computer program for electrodiagnostic examination (EDx) of patients with suspected CTS.

All patients referred to our institution in 2010 with suspected CTS were eligible for inclusion in the study. Our computer program, which guides the examiner through the EDx, was employed. Measurements included median and ulnar motor NCSs and up to 3 median sensory comparison studies. In hands with non-recordable motor or sensory responses, a middle finger sensory study was performed. The sensitivity of individual tests was calculated.

In 2010 the computer program was used in 1935 patients (3870 hands) with suspected CTS. A thumb sensory comparison study was required in 23%, wrist study in 3% and a middle finger study in 19% of hands. The sensitivity of EDx was 45% after motor NCSs and 68% after ring finger, 73% after thumb, 74% after wrist, and 82% after middle finger sensory studies. CTS was confirmed by sensory studies in > 95% hands with a median distal motor latency ≥ 5 ms or amplitude ≤ 4 mV, or a non-recordable ring finger median sensory response.

In the majority of patients only motor NCSs and a single (most often ring finger) sensory comparison study were needed to confirm or exclude CTS. However, additional comparison studies increased the sensitivity of EDx diagnosis of CTS in hands with borderline results.

The study findings were introduced into our current EDx computer program.

Dr. Janez Faganel Memorial Lecture

Biomarkers for myasthenia gravis

Donald B. Sanders

Duke University Medical Center, Durham, USA

A biological marker or biomarker is an objectively measured characteristic that indicates a normal or pathogenic biological process, or reflects a biological response to a therapeutic intervention. Biomarkers are essential in clinical trials to measure disease severity or the therapeutic activity of a treatment under investigation. In myasthenia gravis (MG), potential biomarkers include serum antibodies and neuromuscular jitter.

To assess and compare the potential value of these biomarkers in MG, we reviewed their correlation with disease severity and responsiveness to clinical change in a large population of patients.

Serum antibodies to the acetylcholine receptor or MuSK are found in more than 90% of MG patients, and generally fall with immunosuppressive treatment or after thymectomy. However, the change may be a non-specific response to immunosuppression and frequently does not correlate with clinical change, limiting their role as biomarkers.

Three parameters of jitter measurement (the mean MCD, the percent of pairs with blocking, and the percent of pairs with normal jitter) in the EDC and frontalis each correlate with overall disease severity; measurements in the frontalis discriminate better between remission and ocular disease, and would be preferred as a marker of disease severity.

Calculation of composite Z-scores for these parameters demonstrated that a formula using mean MCD and % normal endplates best reflects change in disease severity.

It is concluded that measurement of jitter is the best pharmacodynamic biomarker for monitoring disease activity in MG. Because the requisite expertise is not widely available, which limits its use in clinical trials, jitter measurement would be most useful in early phase studies.

An unusual presentation of Lambert-Eaton myasthenic syndrome

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[2]Department of Neurology, Southern General Hospital, Glasgow, UK

A 43 year old lady non-smoker was referred by an orthopaedic surgeon for nerve conduction studies (NCS) with symptoms of bilateral carpal tunnel syndrome. She had right carpal tunnel decompression 1 year prior. Examination showed bilaterally reduced pin prick sensation in median distribution.

Normal sensory responses were present in median, superficial radial and ulnar nerves. The motor studies showed small compound motor action potentials (CMAP) in median and ulnar nerves on the right; distal motor latencies were normal. Similar pattern was seen in the right tibial nerve. Ten-second intense isometric exercise test performed in the left abductor digiti minimi and right abductor pollicis brevis showed > 100% increment in CMAP amplitudes following exercise. 3Hz slow repetitive nerve stimulation was performed in the right median nerve showed significant decrement (15.9%) which quickly repaired after intense isometric exercise. A diagnosis of Lambert-Eaton myasthenic syndrome was made on the basis of these findings but clinically she remained well.

Nine months later, she was complaining of proximal limbs weakness, breathlessness on exertion and a dry mouth. Neurological examination revealed reduced power in proximal muscles and absent reflexes in the upper limbs, which improved after facilitation. She did not respond to pyridostigmine or intravenous immunoglobulin but responded to combination of 3,4 diaminopyridine and corticosteroid. Voltage gated calcium channel (VGCC) antibodies remained negative. PET FDG scan showed high uptake in the right lobe of the thyroid gland and the small bowel. She is awaiting further investigations to assess whether these abnormalities represent malignancy.

Lambert-Eaton myasthenic syndrome – repetitive stimulation in 3 patients

Eduard Minks, Ivica Husárová, Hana Streitová, Martin Bareš

First Department of Neurology, St. Anne's University Hospital and School of Medicine, Masaryk University, Brno, Czech Republic

Lambert-Eaton myasthenic syndrome (LEMS) is a rare autoimmune disease with antibodies against voltage-controlled calcium channels (antiVGCC). The etiology can be paraneoplastic or idiopathic. Clinically, the patients have paresis of proximal muscle in the limbs, hyporeflexia, ptosis, fatigue, and autonomic symptoms. Electromyography is essential for diagnosis. The initial amplitude of the compound muscle action potential (CMAP) is strikingly low and is increased after facilitation. Low-frequency repetitive nerve stimulation (LFS) 2-5Hz shows decrement of amplitude and high frequency (HFS) 20-50 Hz shows increment (increment over 100% is diagnostic for LEMS). The aim of the presentation is to share the initial electrophysiological findings in our 3 patients. Age of patients (female) were 30-57 years, the development of disease was 0.2 to 4 years, all of them had positive antiVGCC type P/Q. One patient had a paraneoplastic form. If we stimulated musculus abductor digit minimi (ADM), we found that the initial amplitude of CMAP was low in only one patient. LFS 3Hz showed a decrement of at least 10% just in one patient and HFS 30Hz showed increment 40%, 67% and 600%. If we stimulated musculus abductor hallucis (AH), the initial amplitude of CMAP was low only in one patient, LFS 3Hz showed decrement of more than 10% in two patients and the HFS 30Hz showed increment 117%, 136% and 200%. In conclusion, we consider that AH should be always investigated by HFS 30Hz, where we constantly recorded increment over 100% in contrast to the stimulation of ADM.

Supported by MUNI/A/0897/2010.

Keywords: Lambert-Eaton, myasthenic syndrome, repetitive stimulation, decrement, increment

Effect of bipolar electrostimulation on focal neuropathy of ulnar nerve in elbow area – pilot study

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The focal neuropathy in the elbow area is the second most common mononeuropathy. The goal of our pilot study was to analyze the effectiveness of the bipolar electrostimulation of the ulnar nerve lesion in the elbow, using clinical examination, electromyography, questionnaires, motor tests, and Hoorweg-Weiss I/t curves. Study included 10 patients, 49 ± 14.2 years old, with clinical noninjury, mild paresis of ulnar nerve. Nerve conduction studies revealed mild slowing of MCV, SCV and focal nerve lesion in ulnar nerve sulcus with subacute axonal neuropathy in needle EMG in m.IDI and FCU. Participants received bipolar electrostimulation (oblique pulse at subtreshold motor level, pulse duration 500ms) twice a week for six weeks. A paired t-test was used to compare the input and output EMG values, questionnaires (DASH, UNEQ),and motor tests ($p < 0.05$). The results showed increase in amplitude of CMAP from IDI (10.6 ± 2.9 mV vs. 12.5 ± 3.5 mV, *0.004),and improvements in questionnaires. No changes were found in SNAP from V.finger (9.7 ± 4.2 μV versus 12.1 ± 7.8 μV, *0.36) or in MCV across the elbow from ADM (38.6 ± 10.8 m/s versus 38.7 ± 9.1 m/s, *0.94) and IDI (39.6 ± 13.0 m/s versus 39.1 ± 9.9 m/s, *0.92). I/t curves revealed a nonsignificant increase in accomodative quotient (2.7 ± 0.4 versus 3.3 ± 0.7 * 0.12). While the present study shows nonsignificant impact of the bipolar electrostimulation on focal neuropathy, the subjective improvement and increase in amplitude of CMAP from IDI are indicative of its positive effect.

Keywords: ulnar nerve, bipolar electrostimulation, EMG

Inching in focal neuropathy of the ulnar nerve in the elbow

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Clinical electromyography is a part of the diagnostics of focal neuropathy of the ulnar nerve in the elbow. The aim of this study was to evaluate an usefulness of inching in our laboratory.

Patients: 12 measurements (age 37 years, duration of disease 12 months - median). Clinically all patients expressed mild paresis and atrophy in the hand, paresthesia and hypoesthesia in the distribution of this nerve. Control group: 5 measurements (age 33 years - median). Inching the ulnar nerve in the elbow was made in 2 cm segments with registration electrodes on the musculus interosseus dorsalis primus (IDI), the elbow was held at an angle of 135 degrees.

6cm below the olecranon we have not achieved the maximum response of the muscle in 35%. The stimulation in proximal segments was technically correct. Variability in conduction velocity in 2 cm segments was high in healthy volunteers (from 26 to 100 m/s) too, but patients had at least one value below 26 m/s. Conduction block was present in 2 patients.

Inching (due to variability in conduction velocity) does not fit as a routine technique in primary diagnostics. Precise localisation of lesions in 2 cm segment can be determined by finding the conduction block. To make recommendations for the localisation of lesion according to decreased conduction velocity, the extension of the data is needed (this time we can consider a maximal lesion in the 2 cm segment where velocity is decreased below 26 m/s).

Supported by MUNI/A/0897/2010.

Keywords: inching, ulnar nerve

Median nerve F-wave analysis in patients with carpal tunnel syndrome

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F-waves are motor responses produced by antidromic activation of motor neurons after distal stimuli and are a very useful method for assessing proximal conduction of motor fibers. Carpal Tunnel Syndrome (CTS) has been postulated to cause proximal conduction changes of the median nerve. The aim of this work is to evaluate the median nerve retrograde abnormalities in patients with CTS by studying F-wave changes.

We retrospectively studied 582 patients (13-89 years) with clinical CTS. Median nerve F-waves were evaluated following 20 supramaximal stimuli and recording from APB muscles. We studied the following parameters: minimum, maximum and, mean F wave latency (FMINLAT, FMAXLAT, and FMEANLAT), number of F waves/20 stimuli (FNUMBER), and F wave chronodispersion (FDISP = FMAXLAT - FMINLAT). We compared our neurographic findings with those of the database for healthy subjects established by Puksa et al (2003). T-tests were used for comparison of means (SPSS_v15.0).

Patients with clinical and electrophysiological signs of CTS showed statistically significant differences of FMINLAT, FMAXLAT, FMEANLAT, and FDISP when compared with healthy subjects. In addition, significant differences in FMINLAT, FMEANLAT, and FDISP were found in patients with CTS signs and normal distal neurography.

Finally, FNUMBER was significantly lower in patients with CTS and abnormal neurography. However, no significant differences were seen in patients with only symptoms of CTS.

Patients with electrophysiologic signs of CTS show abnormal proximal median nerve conductions assesed by F-waves.

F-wave may show proximal conduction abnormalities even in patients with signs of CTS and normal transcarpal conductions.

Keywords: carpal tunnel syndrome (CTS), F-waves, focal neuropathies

Results to be expected with stimulation SFEMG in early myasthenia gravis

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At the onset of MG, a fully blown clinical picture may appear within a few days. However, clinicians are increasingly aware that mild weakness and fatigue, e.g. isolated in a few extraocular muscles, manifested as fleeting diplopia or ptosis, may be a hallmark of gradually developing MG. Such patients with minimal symptoms and signs of a few weeks duration are increasingly often referred to EMG laboratories for diagnostic evaluation.

The standard technique of jitter study with axonal microstimulation, known to be highly sensitive in demonstrating dysfunction of neuromuscular transmission, was used as the initial diagnostic test in 249 consecutive MG patients upon their first presentation. A group of 37 patients was studied within 12 weeks (1-12, mean 5.2 weeks) of the onset of their first symptoms and signs.

Two criteria of SFEMG abnormality were used. One was the proportion of neuromuscular junctions (NMJs) showing abnormal jitter with or without blocking (percentage of outliers), and the other was mean jitter of all measured NMJs. A sample of 40-60 NMJs, in a few very abnormal cases about 30 NMJs, was evaluated. The study was performed in the orbicularis oculi, with the addition of the frontalis in a minority of cases. For the sake of consistency, patients with predominantly limb symptoms and SFEMG in a limb muscle were not included. The upper reference limits used were 31 µs for MCD of individual NMJs and 21 µs for mean MCD of all NMJs studied. Up to 2 NMJs having larger jitter with or without blocking in a sample of 60 were accepted as normal.

There was no significant difference in the degree of abnormalities between this and the larger group of 212 patients seen at later times after the onset. Similarly, there was no correlation between duration of symptoms and the degree of SFEMG abnormality within the small group. Rather, the degree of abnormality was correlated with the severity of symptoms and signs.

According to the outlier criterion, none of the patients had a normal jitter study, and none of the 130 patients who turned out not to have MG had an abnormal study. On the other hand, when using the criterion of mean MCD per study there was a small overlap of the MG and non-MG populations.

In conclusion, neuromuscular jitter of orbicularis oculi and the frontalis muscles show high sensitivity and specificity even in the earliest stages of MG, when symptoms are mild and even non-persistent. The degree of abnormality is not correlated to duration of disease. It is more pronounced when symptoms and signs are severe, but quite abnormal jitter study may coexist with clinically mild disease.

Stimulation SFEMG in studies of neuromuscular physiology

Jože Trontelj

Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Slovenia

SFEMG allows the recording of action potentials (SFAPs) from individual muscle fibers with high precision in vivo and in situ. The constant shape of the SFAPs allows for fine time measurement between an event and the depolarization of the responding muscle fiber. Reproducibility of muscle fiber conduction time over a distance of 20 mm may be as high as 100-300 nanoseconds. Measurements of latency variability of consecutive activations (the jitter in broader sense of the term) may reveal events in the impulse pathways.

On direct electrical stimulation of muscle fibers bimodal latency distribution may occasionally be seen. This indicates the existence of discrete low threshold sites along muscle fibers. At such sites, ephaptic transmission of impulses between muscle fibers may occur; it can be associated with jitter < 4 µs ("low jitter"). When activation is at threshold, the jitter may be very large, in the range of several hundred or thousand µs. Both low and large jitter may be seen between single fiber components of a complex repetitive discharge (CRD), which was taken as an indication of their ephaptic origin. The high frequency variety is believed to take a closed loop impulse pathway along adjacent muscle fibers with low threshold sites. The high repetition rate of the CRD is triggered by a burst of extra-discharges in the principal pacemaker fiber. Open loops underlie low frequency CRDs.

A significant jitter may be generated in the muscle fiber due to changing conduction velocity. Part, but not all, of these changes is due to changing length of the muscle fiber during the contraction-relaxation cycle. This has some practical implications in jitter measurement, repetitive nerve stimulation test and motor conduction velocity measurement.

The jitter of a normal neuromuscular junction (NMJ) on axonal stimulation ranges between 5 and 50 µs. It increases in disorders of neuromuscular transmission, during reinnervation and in some myopathies. Scattered abnormality is also seen on the hemiplegic side of stroke patients, consistent with the putative transsynaptic degeneration of lower motoneurons.

The F-responses have only slightly larger jitter compared to the M-responses of the same muscle fibers, the main contribution to the total jitter being generated at the NMJ and in the muscle fiber, due to conduction velocity changes at uneven discharge rate.

The jitter of the monosynaptic H-reflex is roughly between 80 and 200 µs (SD), and that of the first component of the electrically elicited blink reflex is between about 300 and 450 µs, indicating that it has a disynaptic or oligosynaptic pathway. Electrically elicited bulbocavernosus reflex has a small monosynaptic and a larger polysynaptic component.

It is also possible to measure the jitter in the electrically or magnetically activated corticospinal tract responses, however there may be some overlap among the latency distributions of several descending impulses elicited by a single stimulus, the later ones possibly involving longer pathways and/or repetitive firing of corticospinal tract neurons. Jitter studies revealed monosynaptic corticospinal connections to spinal motoneurons innervating both distal and proximal upper limb muscles.

Dr. JANEZ FAGANEL MEMORIAL LECTURES AND SYMPOSIA 1985–2011

1985 **Brain Injury Satellite Symposium – BISS '85**
P. D. Wall (London, UK): Pain mechanisms

1986 **Diagnostics in Neuromuscular Disorders**
K.-G. Henriksson (Linkøping, Sweden): Muscle pain in neuromuscular disorders and primary fibromyalgia

1987 **2nd Yugoslav Symposium on Neurourology and Urodynamics**
J. K. Light (Houston, Texas, USA): Neurogenic bladder in patients with spinal cord injury

1988 **Symposium on Quantitative Electromyography**
E. Stålberg (Uppsala, Sweden): Electromyography – reflection of motor unit's physiology in health and disease

1989 **Symposium on Sensory Encephalography**
A. M. Halliday (London, UK): The widening role of evoked potentials in clinical practice

1990 **Symposium on Assessment of the Upper Motor Neuron Functions**
A. M. Sherwood (Houston, Texas, USA): Brain motor control assessment

1991 **Symposium on Neurophysiological Monitoring**
V. Deletis (New York, NY, USA): Intraoperative monitoring of evoked potentials – current status and perspective

1992 **International Symposium on Evaluation and Treatment of Severe Head Injury**
E. Rumpl (Klagenfurt, Austria): Neurophysiological evaluation of severe head injury patients

1993 **Symposium on Neurophysiological Evaluation of the Visual System**
H. Ikeda (London, UK): Mammalian retinal neurotransmitters – as seen through the eyes of a neurophysiologist

1994 **Symposium on Extrapyramidal Disorders**
J. Jankovic (Houston, Texas, USA): New horizons in dystonia
and
The First Lecture of the Slovene Basal Ganglia Club:
G. Stern (London, UK): Amara lento tempera risu

1995 **Symposium on Multiple Sclerosis**
W. I. McDonald (London, UK): The clinical and pathological dynamics of multiple sclerosis

1996 **Symposium on Update in Neurogenetics**
L. P. Rowland (New York, NY, USA): Molecular genetics and clinical neurology

1997 **Symposium on Cognitive Neuroscience**
G. Barrett (Farnborough, UK): Cognitive neurophysiology, a tool for studying the breakdown of mental processes

1998 **9th European Congress of Clinical Neurophysiology, Ljubljana**
J. Trontelj (Ljubljana, Slovenia): SFEMG – Sensitive optics in space and time

1999 **Symposium on Electrophysiology of Hearing**
A. Starr (Irvine, California, USA): Mysteries of the cochlea

2000 **Symposium on Movement Disorders, "The Alpine Basal Ganglia Club"**
A. J. Lees (London, UK): The relevance of pleasure/reward dopamine circuits to Parkinson's disease

2001 **EC-IFCN Ljubljana 2001 Regional EMG Refresher Course**
E. Stålberg (Uppsala, Sweden): The role of conventional and advanced electromyography in clinical neurology

2002 **International Symposium on Clinical and Electrophysiologic Diagnostics of Epilepsy**
P. Chauvel (Marseille, France): High-resolution electroencephalography in clinical neurophysiology: applications to epilepsy and evoked potentials

2003 **Symposium on Intraoperative Neurophysiology**
V. E. Amassian (New York, NY, USA): Essentials of neurophysiology of the motor system

2004 **Symposium on Sleep Research**
M. Billiard (Montpellier, France): Excessive daytime sleepiness: clinical impression versus final diagnosis

2005 **37th International Danube Symposium for Neurological Sciences and Continuing Education**
T. Prevec (Ljubljana, Slovenia): Sharp or kind stimulus to activate the sensory system?

2006 **International Symposium on Spinal Cord Motor Control "From Denervated Muscles to Neurocontrol of Locomotion"**

G. Vrbová (London, UK): Some observations on the biology of the neuromuscular system and their possible usefulness for recovery of impaired function

2007 **XVIth International SFEMG and QEMG Course and IXth Quantitative EMG Conference**

J. Kimura (Kyoto, Japan): The use of late responses as a quantitative measure of nerve conduction and motor neuron excitability

2008 **Symposium on Amyotrophic Lateral Sclerosis**

P. N. Leigh (London, UK): ALS: Advances in the laboratory and in the clinic

2009 **Symposium on Clinical Neurophysiology of Pain**

G. Cruccu (Rome, Italy): Clinical neuropohysiology of pain

2010 **Symposium on Clinical Neurophysiology of Vision and on Eye Movements**

R. Kakigi (Okazaki, Japan): Face recognition-related potentials: EEG, MEG, NIRS studies

2011 **International Course on EMG, SFEMG and Nerve Ultrasonography**

D. B. Sanders (Durham, North Carolina, USA): Biomarkers for myasthenia gravis

The 2012 International Symposium
with the 28th Dr. Janez Faganel Memorial Lecture
Ljubljana, Slovenia, 5–6 October 2012

INVITATION

Dear Colleagues and Friends,

The International classification of sleep disorders distinguishes more than 80 conditions which – in most cases – can be easily managed once they are properly diagnosed. However, in Slovenia, the prevalence, burden, and management of sleep disorders are too often either overlooked or even ignored by individuals, doctors, and medical authorities in general. Ensuing underappreciation and undertreatment of sleep disorders make this group of illnesses a serious health concern.

The fundamental mission of the Slovenian Sleep Society within the Slovenian Society of Clinical Neurophysiology is to advance sleep health by promoting and encouraging education, research, and patient care among different medical specialists.

It is my pleasure to announce that the 2012 Ljubljana autumn clinical neuroscience meeting, to be held on 5–6 October, will be devoted to sleep medicine and sleep research. It is my great honour to announce the keynote speaker, Professor Claudio Bassetti, Director of Neurocentre of Southern Switzerland, Professor of Neurology at the University Hospital in Zürich, and current President of the European Sleep Research Society. Prof. Bassetti will give the 28th Janez Faganel Memorial Lecture entitled Sleep, sleep disorders and stroke.

I believe the meeting will be a perfect opportunity for professionals to gather new knowledge and share experience from their clinical and research work, while it will importantly contribute to the general awareness that sleep disorders are medical conditions, also demanding prevention, treatment, and research.

Cordially invited to Ljubljana,

Leja Dolenc-Grošelj,
Convenor

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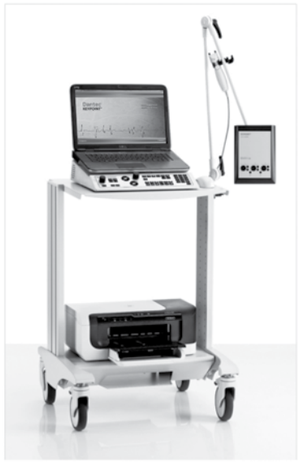

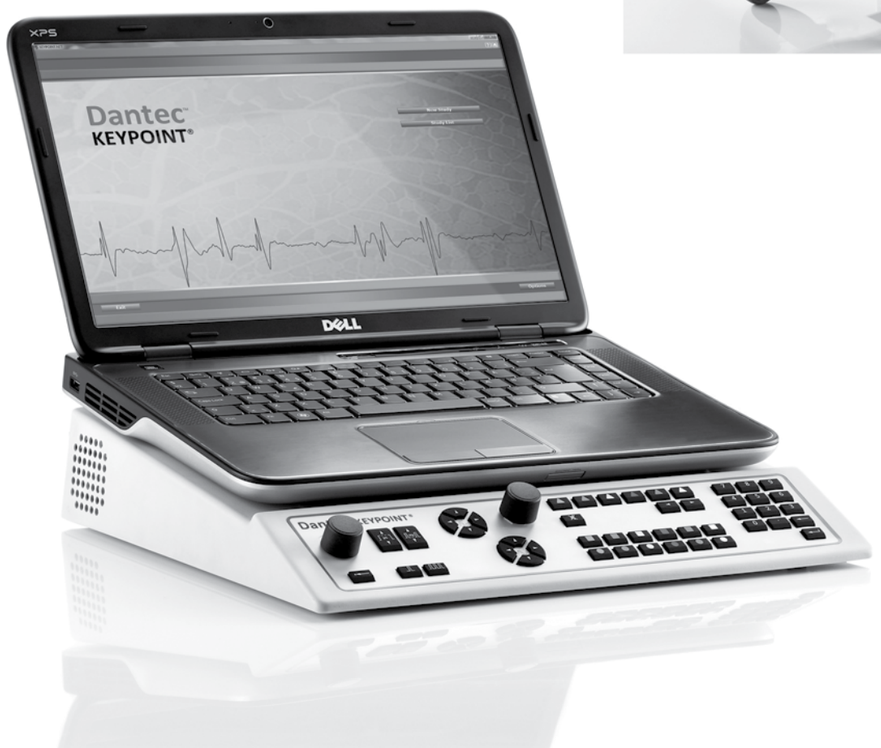

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Galsya® SR

galantamin

kapsule s podaljšanim sproščanjem, 8 mg, 16 mg, 24 mg

Spomnim se...

Galantamin s podaljšanim sproščanjem – za lažje opravljanje vsakodnevnih dejavnosti

Sestava Ena trda kapsula s podaljšanim sproščanjem vsebuje po 8 mg, 16 mg ali 24 mg galantamina (v obliki bromida). **Indikacije** Simptomatsko zdravljenje blage do zmerno hude demence Alzheimerjevega tipa. **Odmerjanje in način uporabe** Zdravilo naj bolnik jemlje enkrat na dan zjutraj, najbolje s hrano. Kapsule je treba pogoltniti cele z nekaj tekočine. Ne sme se jih žvečiti ali zdrobiti. Bolniki, ki imajo težave s požiranjem, lahko kapsule odprejo in pogoltnejo celo tabletno jedro (oz. dve ali vsa tri tabletna jedra) z nekaj tekočine. Vsebine kapsul (tabletnih jeder) se ne sme žvečiti ali zdrobiti. Med zdravljenjem je treba zagotoviti zadosten vnos tekočine. Priporočeni začetni odmerek je 8 mg na dan v obdobju 4 tednov. Začetni vzdrževalni odmerek je 16 mg na dan. Bolnikom je treba ta odmerek dajati najmanj 4 tedne. Po ustreznem preverjanju, ki vključuje oceno kliničnega izboljšanja in prenašanja zdravila, lahko pri posameznih bolnikih pride v poštev povečanje vzdrževalnega odmerka na 24 mg na dan. Če posamezni bolniki odmerka 24 mg na dan ne prenašajo dobro ali če ne pride do kliničnega izboljšanja, je treba razmisliti o zmanjšanju odmerka na 16 mg na dan. **Kontraindikacije** Preobčutljivost za zdravilno učinkovino ali katerokoli pomožno snov. Ker ni podatkov o uporabi galantamina pri bolnikih s hudo jetrno okvaro (več kot 9 po Child-Pughovi lestvici) in hudo ledvično okvaro (kreatininski očistek manjši od 9 ml/min), je galantamin pri teh skupinah bolnikov kontraindiciran. Uporaba galantamina je kontraindicirana tudi pri bolnikih, ki imajo hkrati hujšo ledvično in jetrno okvaro. **Posebna opozorila in varnostni ukrepi** Bolniki z Alzheimerjevo boleznijo hujšajo. Zdravljenje z zaviralci holinesteraze, vključno z galantaminom, je bilo pri teh bolnikih povezano z izgubo telesne mase, zato jo je treba med zdravljenjem nadzorovati. *Srčne bolezni* Zaradi farmakološkega delovanja lahko holinomimetiki vagotonično delujejo na srčno frekvenco (povzročijo npr. bradikardijo). Možnost tovrstnega delovanja je lahko še posebej pomembna pri bolnikih s sindromom bolezni sinusnega vozla ali z drugimi supraventrikularnimi motnjami prevajanja oziroma pri tistih, ki sočasno jemljejo zdravila, ki zelo upočasnijo srčno frekvenco, kot so digoksin in zaviralci adrenergičnih receptorjev beta, ter pri bolnikih s porušenim elektrolitskim ravnovesjem (npr. s hiperkaliemijo ali hipokaliemijo). Pri predpisovanju galantamina bolnikom s srčno-žilnimi boleznimi, npr. takoj po miokardnem infarktu, pri novo odkriti atrijski fibrilaciji, srčnem bloku druge ali višje stopnje, nestabilni angini pektoris ali kongestivnem srčnem popuščanju, še posebej pri skupinah III in IV po klasifikaciji NYHA (New York Heart Association), je potrebna previdnost. *Bolezni prebavil* Pri bolnikih, pri katerih obstaja povečano tveganje za nastanek peptičnih razjed, npr. pri bolnikih z ulkusno boleznijo v anamnezi ali bolnikih, ki so nagnjeni k tem boleznim, vključno s tistimi, ki sočasno jemljejo nesteroidna protivnetna zdravila (NSAID), je treba spremljati pojavljanje simptomov. Uporaba galantamina ni priporočljiva pri bolnikih z obstrukcijo prebavil ali pri bolnikih, ki okrevajo po kirurškem posegu na prebavilih. *Bolezni živčevja* V redkih primerih lahko povečanje holinergičnega tonusa poslabša simptome Parkinsonove bolezni. *Bolezni dihal, prsnega koša in mediastinalnega prostora* Holinomimetike je treba previdno predpisovati bolnikom, ki imajo v anamnezi hudo astmo, obstruktivno pljučno bolezen ali aktivne okužbe pljuč (npr. pljučnico). *Bolezni sečil* Uporabe galantamina ne priporočamo pri bolnikih z zastajanjem seča ali pri tistih, ki okrevajo po kirurškem posegu na sečnem mehurju. **Medsebojno delovanje z drugimi zdravili in druge oblike interakcij** Galantamina zaradi njegovega mehanizma delovanja ne smemo dajati sočasno z drugimi holinomimetiki (npr. z ambenonijem, donepezilom, neostigminom, piridostigminom, rivastigminom ali sistemsko uporabljenim pilokarpinom). Galantamin lahko zavira delovanje antiholinergičnih zdravil. Ob nenadni prekinitvi zdravljenja z antiholinergičnimi zdravili, npr. z atropinom, obstaja tveganje za povečanje učinka galantamina. Kot pri drugih holinomimetikih je možna farmakodinamična interakcija z zdravili, ki zelo zmanjšajo srčno frekvenco, npr. z digoksinom, antagonisti adrenergičnih receptorjev beta, nekaterimi zaviralci kalcijevih kanalčkov in amiodaronom. Previdnost je potrebna pri zdravilih, ki lahko povzročijo *torsades de pointes*. V takih primerih je treba razmisliti o EKG-ju. Galantamin kot holinomimetik lahko okrepi mišično relaksacijo sukcinilholinskega tipa med anestezijo, še posebej pri pomanjkanju psevdoholinesteraze. Sočasno jemanje s hrano upočasni hitrost absorpcije galantamina, ne vpliva pa na njen obseg. Da bi zmanjšali holinergične neželene učinke, priporočamo jemanje zdravila Galsya SR s hrano. Na začetku zdravljenja z močnimi zaviralci CYP2D6 (npr. s kinidinom, paroksetinom, fluoksetinom ali fluvoksaminom) ali CYP3A4 (npr. s ketokonazolom ali ritonavirjem) so pogostejši holinergični neželeni učinki, zlasti slabost in bruhanje. V takih primerih lahko bolniku po potrebi zmanjšamo vzdrževalni odmerek galantamina; odvisno od tega, kako ga prenaša. **Plodnost, nosečnost in dojenje** Za galantamin ni na voljo kliničnih podatkov od nosečnic, ki so bile izpostavljene zdravilu. Pri doječih materah raziskave niso bile narejene, zato ženske, ki se zdravijo z galantaminom, ne smejo dojiti. **Vpliv na sposobnost vožnje in upravljanja s stroji** Galantamin blago do zmerno vpliva na sposobnost za vožnjo in upravljanje strojev. Simptomi vključujejo omotico in zaspanost, zlasti v prvih tednih zdravljenja. **Neželeni učinki** Zelo pogosta neželena učinka, o katerih so poročali, sta bila slabost in bruhanje, ki sta se pojavila predvsem v obdobju titracije odmerka. Pogosti neželeni učinki so bili zmanjšanje teka, anoreksija, halucinacije, depresija, sinkopa, omotica, tremor, glavobol, somnolenca, letargija, bradikardija, hipertenzija, bolečina v trebuhu, driska, dispepsija, nelagodje v želodcu in trebuhu, povečano potenje, mišični krči, utrujenost, astenija, slabo počutje, padci. Ostali neželeni učinki se pojavijo občasno ali redko. **Način izdajanja zdravila** Samo na zdravniški recept. **Oprema** 28 trdih kapsul s podaljšanim sproščanjem po 8 mg, 16 mg in 24 mg galantamina. **Datum priprave besedila** Julij 2011.

Samo za strokovno javnost.

Pred predpisovanjem preberite celoten povzetek glavnih značilnosti zdravila. Objavljen je tudi na www.krka.si.

Slovenija, 8/2011, 2011-14835, SM.

Krka, d. d., Novo mesto, Šmarješka cesta 6, 8501 Novo mesto, www.krka.si

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Svojo inovativnost in znanje posvečamo zdravju. Zato odločnost, vztrajnost in izkušnje usmerjamo k enemu samemu cilju – razvoju učinkovitih in varnih izdelkov vrhunske kakovosti.