International Conference in Neuroscience and Pharmacology

"Neurodegenerative diseases and new trends in pharmacotherapeutic strategies"

April 23-24, 2015

ICNP 2015

RIGA, LATVIA

ABSTRACT BOOK

Baltic-American Freedom Foundation

TIMETABLE

Thursday, April 23

09.00 – 10.00	Registration and coffee
10.00 – 10.30	Opening ceremony
10.30 – 12.10	Session I
12.10 – 13.00	Lunch break
13.00 – 14.15	Session II
14.15 – 14.30	Coffee break
14.30 – 16.35	Session III
16.45	Welcome party

Friday, April 24

9.30 – 11.20	Session IV
11.20 – 11.40	Coffee break
11.40 – 12.40	Poster Session
12.40 – 13.00	Closing ceremony

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WELCOME LETTER

It is our privilege and pleasure to welcome you to the International Conference in Neuroscience and Pharmacology "Neurodegenerative diseases and new trends in pharmacotherapeutic strategies" (ICNP 2015) taking place in Riga.

ICNP 2015 has been organized by the University of Latvia, Faculty of Medicine, Department of Pharmacology and the Latvian Society of Pharmacology.

This event was made possible by funding from the Baltic-American Freedom Foundation (BAFF). For more information about BAFF scholarships and speaker support, visit www.balticamericanfreedomfoundation.org

The main goal of the conference - to bridge basic and clinical research in Neuroscience and Pharmacology between the three Baltic States and experts from the United States of America.

The emphasis of the meeting will be put on different cellular regulatory systems as novel drug targets for application in Neurodegenerative disease therapy.

We are thrilled to present a scientific program covering different aspects of Neuroscience and Pharmacology in lectures of invited speakers and in the poster session on the second conference day.

We are especially excited to have with us participants from each of the Baltic States and from the United States of America and we really hope that the interaction between the participating sides will stimulate further exchange in expertise and will promote new, exciting collaboration perspectives.

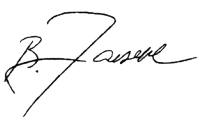
We also wish you an enjoyable stay in Riga, cultural capital of Europe 2014 and the first half year of 2015 the hosting city for the Presidency of the Council of the European Union.

The event was supported also by GenMedica and Grindex.

On behalf of Local Organizing Committee: Mg. biol. Ulrika Beitnere

On behalf of ICNP 2015 International Scientific Programme Committee:
Prof. Vija Zaiga Klusa

On behalf of the Latvian Society of Pharmacology: Head, Dr. Baiba Jansone



COMMITEES

Organizational committee:

Chair: Mg. biol. Ulrika Beitnere (Latvia)

Prof. Vija Zaiga Klusa (Latvia)

Assoc. Prof. Baiba Jansone (Latvia)

Dr. Inga Kadish (USA)

Prof. Ruta Muceniece (Latvia)

Prof. Ago Rinken (Estonia)

Dr. Maija Dambrova (Latvia)

Dr. Ingrida Rumba-Rozenfelde (Latvia)

Poster Award committee:

Dr. Inga Kadish (USA)

Prof. Ruta Muceniece (Latvia)

Dr. Peteris Alberts (Sweden/Latvia)

Special thanks to coordinators and helpers:

Vladimirs Pilipenko, Paula Beitnere, Ineta Popena, Diana Strausa, Jolanta Pupure, Zane Dzirkale, Kaspars Jekabsons and many others

GENERAL INFORMATION

Dates

April 23-24, 2015

Conference venue

University of Latvia
Raina Blvd. 19
Small hall, 2nd floor

Registration desk

Foyer of Small Hall, 2nd floor

April 23, 9.00 - 10.00

Upon registration you will receive:

- Name badge (you are kindly requested to wear your badge during all conference sessions and events)
- Program/Abstract book

Certificate of attendance

Certificates of attendance will be available on the first day afternoon till the end of the conference at the registration desk. Please ensure that you have your name badge with you as you need to show it as a proof of registration

Coffee breaks

Will be served in the designated areas as per schedule

Language

English

Posters display

Please check the Abstract book for the board number on which you should display your poster(s). Posters should be mounted between 9.00-10.00 on Thursday, April 23 and removed by the end of the closing ceremony Friday, April 24

Poster presenters should plan to be next to their poster board during the poster session

ACKNOWLEDGEMENTS

The conference is funded by the Baltic-American Freedom Foundation (BAFF), GenMedica, Grindex, Adrona, EPHAR, University of Latvia, and organized by the University of Latvia, Faculty of Medicine and the Latvian Society of Pharmacology.

Baltic-American Freedom Foundation

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EPHAR

THE FEDERATION OF EUROPEAN PHARMACOLOGICAL SOCIETIES

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ANNO 1919

UNIVERSITY OF LATVIA

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SCIENTIFIC PROGRAM

THURSDAY, APRIL 23

09.00 - 10.00 Registration and coffee

10.00 – 10.30 Opening ceremony
Welcome Address:
Ulrika Beitnere (Executive Organizer, University of Latvia, Latvia)
Marcis Auzins (Rector, University of Latvia)
Ilze Doskina (Regional Manager, BAFF)
Ingrida Rumba-Rozenfelde (Dean, University of Latvia)

Session I Chair: Maija Dambrova (Latvia), Thomas van Groen (USA)

10.30 – 10.55 L1 **How neuropathologic observations have guided Alzheimer research since the 1960s**
Harry Vinters (University of California, Los Angeles, USA)

10.55 – 11.20 L2 **Ghrelin as Alzheimer's disease modifying peptide**
Inga Kadish (University of Alabama, Birmingham, USA)

11.20 – 11.45 L3 **Alzheimer's disease and depression - two entities of the same disease? New treatment options for neurodegenerative diseases using ABC transporter.**
Jens Pahnke (University of Oslo, Norway)

11.45 – 12.10 L4 **Does memantine in combination with melatonin affect disease progression in the animal models of Alzheimer's disease? New challenges with old drugs**
Aleksander Zarkovski (Tartu University, Estonia)

12.10 – 13.00 Lunch break

Session II Chair: Baiba Jansone (Latvia), Ivars Kalviņš (Latvia)

13.00 – 13.25 L5 **Cognitive analysis in rodent neurological disease models**
Thomas van Groen (University of Alabama, Birmingham, USA)

13.25 – 13.50 L6 **Neurodegenerative diseases: models, problems, drugs, strategies**
Vija Kluša (University of Latvia, Latvia)

13.50 – 14.15 L7 **Bringing 3D Tomographic in-vivo animal imaging to LIFE**
Jurijs Dmitrijevs (GenMedica, Latvia)

14.15 – 14.30 Coffee break

Session III	Chair: Vija Kluša (Latvia), Una Riekstiņa (Latvia)
14.30 – 14.55	L8 **Discovery of cognition enhancer E1R, a novel positive allosteric modulator of sigma-1 receptors** *Maija Dambrova (Latvian Institute of Organic Synthesis, Latvia)*
14.55 – 15.20	L9 **Fluorescence-based methods for characterization of ligand binding to GPCR** *Ago Rinken (Tartu University, Estonia)*
15.20 – 15.45	EPHAR sponsored lecture: L10 **Treating the brain with diabetes drugs in Alzheimer's disease** *Jørgen Rungby (Aarhus University, Denmark)*
15.45 – 16.10	EPHAR sponsored lecture: L11 **Identification of novel genes with Alzheimer's Disease by genomic-functional analysis in a HSV-1 infection cell model of neurodegeneration** *Henrike Kristen (Center for Molecular Biology „Severo Ochoa", Spain)*
16.10 – 16.35	L12 **Studies of the BRICHOS domain, insights into an anti-amyloid chaperone** *Henrik Biverstål (Karolinska Institutet, Sweden)*
16.45	Welcome party

FRIDAY, APRIL 24

Session IV	Lectures Chair: Ago Rinken (Estonia), Augustas Pivoriunas (Lithuania)
9.30 – 9.55	L13 **Modifications of NO production in brain by halogenated volatile anesthetics and natural compounds** *Nikolajs Sjakste (University of Latvia)*
9.55 – 10.20	L14 **Human dental pulp stem cells as promising tools for neuroregeneration** *Augustas Pivoriunas (State Research Institute Centre for Innovative Medicine, Lithuania)*
10.20 – 10.45	L15 **Exosomes from dental pulp stem cells rescue human dopaminergic neurons from 6-hydroxy-dopamine-induced apoptosis** *Akvile Jarmalaviciute (State Research Institute Centre for Innovative Medicine, Lithuania)*
10.45 – 11.10	L16 **Neuroglial properties of skin-derived mesenchymal stem cells and development of in vitro model for drug testing** *Una Riekstiņa (University of Latvia, Latvia)*

11.10 – 11.20	**Baltic American Freedom Foundation programs and a year of research in the U.S.** *Ulrika Beitnere (University of Latvia)*
11.20 – 11.40	Coffee break
11.40 – 12.40	**Poster session** Chair: Inga Kadish (USA), Ruta Muceniece (Latvia), Pēteris Alberts (Sweden/Latvia)
12.40 – 13.00	**Poster Award Announcement**
	Closing Ceremony

ABSTRACTS

L1

HOW NEUROPATHOLOGIC OBSERVATIONS HAVE GUIDED ALZHEIMER RESEARCH SINCE THE 1960s

Harry Valdis Vinters

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Alzheimer's disease is characterized, from a neuropathologic perspective, by brain atrophy accompanied by the accumulation within the CNS (especially cerebral cortex) of characteristic 'lesions'—these include senile (neuritic) plaques (SPs), neurofibrillary tangles (NFTs), neuropil threads (nt), and granulovacuolar degeneration (GVD). SPs have a major component of ABeta (beta-amyloid) in their cores, and tau within their neuritic 'halos'; NFTs contain phospho-tau. Whereas SPs, NFTs and nt's are found in both hippocampus and neocortex, GVD is, in most individuals, confined to the hippocampal pyramidal cell layer, where it may be quite pronounced and may reflect an abnormality of autophagy. A microvascular lesion (cerebral amyloid/congophilic angiopathy, CAA) results from ABeta amyloid protein accumulating within the walls of cerebral and meningeal arterioles and capillaries; this microvasculopathy may result in lobar cerebral hemorrhages, microbleeds, and microinfarcts within the brain.

Biochemical and immunohistochemical examination of autopsy brain specimens from AD/SDAT patients has provided much of the framework on which mechanistic studies of AD pathogenesis are performed. The goal of many transgenic (Tg) mouse studies of AD has been to replicate AD lesions in the brains of these animals, and look for correlations with neurobehavioral abnormalities. The neuropathologic staging of AD has also evolved in recent decades. The Khachaturian, CERAD and Braak approaches to staging of AD neuropathologic change has been incorporated into a recently proposed scheme, initially described as the "ABC" approach (Montine et al., 2012).

Neuropathologists have recognized for many decades that there may be significant 'Alzheimerization' of the brain in the absence of cognitive impairment—a finding that is now replicated in antemortem studies of amyloid accumulation within the brain using various PET ligands (PiB, FDDNP, Amivid).

As therapies for AD/SDAT are developed, neuropathologic studies will be crucial in helping to explain why various treatments are effective or not effective in preventing or curing this common form of neurodegeneration.

Acknowledgements. HVV supported in part by NIA P50 AG 16570 grant.

L2

GHRELIN AS ALZHEIMER'S DISEASE AND AGING MODIFYING PEPTIDE

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Caloric restriction (CR) is a long established paradigm, which extends longevity and slows symptoms of aging through mechanisms which have yet to be clearly elucidated. Ghrelin is a hunger-inducing gut peptide, and the interoceptive cues caused by ghrelin are likely similar to those produced by CR. We tested the novel hypothesis that a ghrelin agonist would attenuate behavioral and cognitive decline in Alzheimer's disease (AD) model mice. Further, we tested whether it would change energy metabolism and circadian rhythm in aged SAMR1/SAMP8 mice and that these changes involve interoceptive cues, rather than reduced energy intake per se.

In an AD mouse model, four month treatment with a ghrelin agonist was sufficient to improve the performance in the water maze and to reduce the levels of amyloid beta (Aβ) and inflammation (microglial activation) at 6 months of age compared to the control group, similar to the effect of CR. In the aging study two groups of SAMR1 and SAMP8 male of 2 month old mice were used in this study. One group (control) from each strain had ad libitum access to food, while the second group (ghrelin) received the mean amount of diet consumed by the control group and a ghrelin agonist (LY444711; 30 mg/kg of LY in a 45 mg sucrose pellet) daily for 6 months.

Cognition was significantly improved in ghrelin group of SAMR1 mice compared to control group, but did not influence the cognitive outcome of SAMP8 mice. In a 12:12 light-dark cycle, both SAMR1 and SAMP8 mice exhibited typical 24-hour rhythmicity in general cage activity, respiratory exchange ratio (RER), and energy expenditure; however, SAMP8 mice exhibited a pronounced ultradian (12-hour) rhythm in all measurements as well. Daily ghrelin administration to SAMP8 mice eliminated the ultradian phenotype and restored the amplitude of 24-hour rhythms in behavior and metabolism up to the level seen in SAMR1 controls.

In conclusion, "hunger" without caloric restriction has similar cognitive and metabolic benefits to caloric restriction, without the potential problem of weight loss in aging patients.

L3

ALZHEIMER'S DISEASE AND DEPRESSION – TWO ENTITIES OF THE SAME DISEASE? NEW TREATMENT OPTIONS FOR NEURODEGENERATIVE DISEASES USING ABC TRANSPORTER

Jens Pahnke

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In elderly subjects, depression and dementia often coincide but the actual reason is currently unknown. Does a causal link exist or is it just a reactive effect of the knowledge to suffer from dementia? The ABC transporter superfamily may represent a causal link between these mental disorders. Does the treatment of depression in elderly subjects using pharmacological compounds or phytomedical extracts target a mechanism that also accounts for peptide storage in Alzheimer's disease and perhaps other proteopathies of the brain?

In this talk I will summarize recent data that point to a common mechanism and present the first promising causal treatment results of demented elderly subjects.

L4

THE ANALYSIS OF THE EFFICACY OF THE COMBINATION OF MEMANTINE WITH MELATONIN IN THE ANIMAL MODELS OF ALZHEIMER'S DISEASE: NEW CHALLENGES WITH FAILED DRUGS

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Background. Memantine was introduced into clinical practice in Europe in 2002 as a drug for the treatment of Alzheimer's disease (AD). According to the current concept, the effect of memantine is linked to its neuroprotective effect, i.e. the ability to protect nerve cells from death (Kutzing et al., 2011). Recently the efficacy of memantine was questioned even when the drug was used in patients with mild AD (Schneider et al., 2011). Another drug, which might have a potential in the treatment of AD is melatonin. Several studies have showed that melatonin levels are diminished in AD patients compared to age-matched control subjects. CSF melatonin levels decrease even in preclinical stages when the patients do not manifest any cognitive impairment that the reduction in melatonin is an early marker for the first stages. The attempts to use melatonin to correct sleep disturbances in AD patients did not provide clear evidence for its efficacy in AD patients (Singer et al, 2003). The aim of this experiment study was to evaluate whether combination of memantine with melatonin would be more efficacious than drugs given alone with respect of their neuroprotective and cognition improving actions.

Methods. The effects of the combination of memantine with melatonin were studied in (i) in vitro models of neurototoxicity on the primary neuronal cultures of the cerebellar granule cells, (ii) on the in vivo model of impaired cognitive functions after intracerebroventicular administration of beta amyloid peptide fragment (AB25-35) in mice and (iii) the transgenic 5xFAD mice bearing five mutations in amyloid precursor protein and presenilin genes. Results. In the primary culture of the cerebellar granule cells, the addition of memantine with melationin in concentrations 1.0 + 0.6 uM and 10 +6 uM demonstrated highly neuroprotective effects against toxicity induced by AB25-35, glutamate, kainic acid. In in vivo experiments, memantine + melatonin (5+3 mg/kg and 10+6 mg/kg) exerted anti-amnestic affect and reduced neurodegeneration induced by the intracerebroventicular administration of AB25-35. This combination given during repeatedly during 30 days in 5xFAD transgenic mice reduced the density of the amyloid plagues and the signs of the neuro-inflammation in the brain of the transgenic mice. This effect was accompanied with the episodic memory improvement (object recognition test). In all experiments, the efficacy of the combination was significantly higher as compared with memantine or melatonin given alone.

Conclusions. The obtained data demonstrate higher efficacy of the combination of memantine with melatonin in the animal models for AD. It seems to be necessary to re-evaluate the efficacy of this combination in the clinical settings keeping in mind appropriate end-points.

Acknowledgements. This study was supported by Estonian Science Council Grant (Institutional research founding) IUT23, Archimedes Foundation and European Regional Development Fund.

L5

COGNITIVE ANALYSIS IN RODENT NEUROLOGICAL DISEASE MODELS

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The goal of Cognitive Behavioral Assessment is to do behavioral testing procedures for rodent models of neurological diseases. To do this, we need to provide a broad array of state-of-the-art behavioral tools to understand behavioral/cognitive phenotypes and rodent cognition. We need to work systematically towards (i) test diversity, (ii) ethologically reasonable and the least stressful behavioral assays, (iii) increased automation (easier to handle, and more reproducible) and higher throughput testing.

Transgenic and gene-targeting approaches in mice (and rats) are increasingly providing detailed characterization of mechanisms underlying behavior in normal and diseased states. Defining the behavioral phenotype of a mutation is very important but we need to conduct sophisticated, rigorous testing of behavioral phenotypes in these animals.

Further complicating the interpretation of these studies is the finding that there are marked differences in behavioral phenotype between strains of inbred mice that are typically used as the background for genetic models of disease.

Novel tools for behavioral diagnostics need to be continuously added to the set of testing systems that are available. We continue to develop (semi-)automated equipment and analytical tools for large animal behavior datasets. We try to develop (i) more test diversity, (ii) ethologically reasonable and, thus, putatively less stressful behavioral assays, (iii) increased automation potentially leading to high throughput testing. A knowledge database of testing procedures for animal models of neurological and psychiatric function needs to be available.

L6

NEURODEGENERATIVE DISEASES: PROBLEMS, DRUGS, PHARMACOTHERAPEUTIC STRATEGIES

Vija Klusa[1], Ulrika Beitnere[1], Vladimirs Pilipenko[1], Jolanta Pupure[1], Juris Rumaks[1], Simons Svirskis[1], Baiba Jansone[1], Ruta Muceniece[1], Zane Dzirkale[1], Sergejs Isajevs[2], Thomas van Groen[3], Inga Kadish[3]

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Background. Neurodegenerative diseases are associated with misfolded protein aggregates, however, it has been shown that these proteins start to disrupt synapses leading to cognitive decline before the onset of protein aggregation. As a result neurodegenerative diseases are seen as a consequence of degenerating synapses, likely leading to an imbalance of excitation and inhibition. Furthermore, the synthesis of neurotrophic factors crucial for neurogenesis is reduced. We suggest that early multi-targeting directed to maintain cell survival and plasticity, may prevent brain deterioration and, thus, slow mental decline. The novel focus of the present studies was on the GABAergic system because of the reduced GABA transmission in human Alzheimer's disease (AD) brains correlated with decline in memory, anxiety and depression. Methods. We have chosen two small molecules: carnitine congener mildronate (50 and 100 mg/kg, ip, 2-4 weeks) tested in Parkinson's disease (PD) model-rats (intrastriatal 6-OHDA) and AD transgenic (Tg) APPSweDI mice; 1,4-dihydropyridine derivative AP-12 (0.1 and 1 mg/kg ip, 3 weeks) tested in Tg mice. In addition, muscimol (0.01 and 0.05 mg/kg ip, 7 days), GABA-A receptor agonist was assessed in streptozocin (STZ, icv) AD model-rats. Animals' behavior was evaluated in water maze and/or elevated plus maze. Protein expression in the hippocampus and/or striatum was assessed immunohistochemically and by Western bloting. Mitochondrial processes were assessed in isolated mitochondria, as well as in azidothymidine model mice.

Results. Mildronate in PD model protected 6-OHDA-induced loss of tyrosine hydroxylase (TH)-positive nerve endings, increased number of progenitor Notch-3-, nerve cell adhesion molecule (NCAM)-, glial derived neurotrophic factor (GDNF)- and molecular chaperon HSP70-positive cells. In Tg mice, mildronate caused acetylchiline esterase (AchE) downregulation, and lowered amyloid-β deposition in the hippocampus. In trained rats, mildronate increased the number of BrdU/nestin-positive cells in the hippocampus. Mildronate also restored compromized mitochondrial functions, normalized micro-and macroglial activities. AP-12 induced glutamic acid decarboxylase (GAD67) upregulation and showed memory-stimulating and anxiolytic actions in Tg mice. Muscimol significantly improved spatial memory in STZ-model rats.

Conclusions. Mildronate stimulates brain processes involved in synaptic plasticity, protects mitochondrial functions and shows anti-inflammatory action. The mechanism of AP-12 action to a great extent involves GABAergic component. The role of GABA mechanisms in spatial memory was confirmed also by muscimol administration in AD model-rats.

Acknowledgements. ESF project No. 2009/0217/1DP/1.1.1.2.0/09/APIA/VIAA/031; LZP grant Nr. 09.1025; NIH P30 NS47466.

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L7

BRINGING 3D TOMOGRAPHIC IN-VIVO ANIMAL IMAGING TO LIFE

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The rapid development in medical research produces a continuous stream of new knowledge about disease processes, new therapeutic targets and the complex relationship between a person's genome and his/her related risk for disease. New technology is being developed for all aspects of patient care and the potential benefits of personalized medicine combined with medical imaging technologies is gaining acceptance. In particular, Medical Imaging plays a central role in the global healthcare system as it contributes to improved patient outcome and more cost-efficient healthcare for all major diseases.

However, while Medical Imaging traces its technology roots to the 1950's since the invention of the Anger camera, and while it has continued to evolve, the underlying principle of operation of the detector has not changed during this time. This has set limits on the performance that can be obtained with nuclear and optical cameras.

The commercial availability of digital CZT (Cadmium Zinc Telluride), APD (Avalanche Photon Diode) and Digital Single-Photon (Digital Si-PM) detector arrays has allowed nuclear and optical camera designers to challenge many of the traditional design constraints. The result is a new generation of Nuclear Medicine and Optical Imaging equipment with previously unheard of performance and clinical utility.

In this presentation, we will highlight the impact of these new digital detection technologies on preclinical imaging, the discipline used to image small animals such as mice – more than 2000 times smaller than humans - with the same visual acuity as is possible with current non-digital technologies for imaging humans. These highly performing and compact imaging technologies can be translated to the clinic so that in the near-term, more and better research in medical imaging at lower costs can be employed to increase our knowledge about disease processes and therapy management with the long-term goal of improving the health of the global population.

During the presentation, we will highlight the commercial and research implications of this digital detection technology revolution, and illustrate with real-world application examples the effect that these techniques may have on accelerating our knowledge about disease processes and the discovery of potential treatments for new therapeutic targets.

L8

DISCOVERY OF COGNITION ENHANCER E1R, A NOVEL POSITIVE ALLOSTERIC MODULATOR OF SIGMA-1 RECEPTORS

Maija Dambrova[1, 2], Edijs Vavers[1,2], Baiba Svalbe[1], Ilga Misane[3], Liga Zvejniece[1]

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Background. The sigma-1 receptor (Sig1-R) is a chaperone protein that modulates intracellular calcium signalling of the endoplasmatic reticulum. Several lines of evidence suggest that the Sig1-R agonists are effective in treatment of cognitive impairments in experimental animal models. We discovered a novel 4,5-disubstituted derivative of piracetam E1R ((4R,5S)-2-(5-methyl-2-oxo-4-phenyl-pyrrolidin-1-yl)-acetamide), which acts as a positive allosteric modulator of Sig1-R and enhances cognition and memory processes in mice.

Methods. The mechanism of action of E1R was characterized in [3H](+)-pentazocine binding and bradykininduced intracellular Ca2+ concentration ([Ca2+]i) increase assays. The effects of E1R on cognition were evaluated using passive avoidance (PA) and Y-maze tests. Because Sig1-R agonists potently modulate acetylcholine release, scopolamine-induced amnesia was used as an experimental model for the memory impairment caused by cholinergic dysfunction.

Results. E1R did not displace [3H](+)-pentazocine from the Sig1-R, but the pre-incubation with E1R potentiated 3 times the effect of selective Sig1-R agonist PRE-084 on the [Ca2+]i changes, thus confirming the Sig1-R positive allosteric modulator effect in vitro. The effect of E1R was blocked by treatment with the selective Sig1-R antagonist NE-100. In PA test the acute pre-treatment with E1R facilitated PA retention in a dose-related manner, the effect being statistically significant ($p<0.05$) at doses of 1 and 10 mg/kg. In the PA and Y-maze tests in mice E1R alleviated the scopolamine-induced cognitive impairment. The effect of E1R on scopolamine-induced cognitive deficit was antagonized by the administration of the selective Sig1-R antagonist NE-100. In addition, E1R did not affect locomotor activity in the open-field, rota-rod, traction and cylinder tests.

Conclusions. E1R is a novel 4,5-disubstituted derivative of piracetam, which possesses significant cognition enhancing properties and efficacy against scopolamine-induced cholinergic dysfunction in mice. These effects of E1R are related to its positive modulatory action on Sig1-Rs which might be of interest in developing new drugs for treatment of cognitive symptoms of neurodegenerative diseases.

Acknowledgements. This research was financed by the European Regional Development Fund, Project No. 2010/0237/2DP/ 2.1.1.1.0 / 10 / APIA/VIAA/ 059 and by grant from the Latvian Council of Science (108/2013). JSC Grindeks was a co-financing industrial partner in this project.

L9

FLUORESCENCE-BASED METHODS FOR CHARACTERIZATION OF LIGAND BINDING TO G PROTEIN COUPLED RECEPTORS

Ago Rinken[1, 2], Anni Allikalt[1, 2], Sergei Kopanchuk[1, 2], Reet Link[1], Santa Veiksina[1]

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Background. G protein coupled receptors (GPCR) comprise a large family of transmembrane proteins involved in regulation of signal transduction through the cell membrane in response to various extracellular stimuli and have become important targets of many drugs for treatments of very different diseases. During the last decade several fluorescence-based methods have implemented for characterization of signal transduction via GPCR, starting from ligand binding and including several steps, which lead till responses on gene regulation level.

Methods. We have implemented fluorescence anisotropy (FA) and fluorescence intensity (FI) assay to investigate ligand binding properties to different GPCR (Veiksina et al., 2010) We have used commercially available as well custom synthesized fluorescent dye-labelled ligands. We have used budded baculoviruses as a source of recombinant proteins (Veiksina et al., 2014) of different GPCRs studied.

Results. Developed assay systems opened possibilities for real-time monitoring of ligand binding to their receptors. This would allow to generate HTS homogenous assay systems for ligand screening, but also to understand particular kinetic properties of ligands. Following the change of fluorescence anisotropy in time gives information about the reaching of equilibrium for each particular ligand. However, the interpretation of data of these experiments is more complex than in the case of conventional radioligand binding assays, as here several addition factors may play crucial role, including receptor concentration, ligand depletion, but also modulation of measurable parameters FI and FA by different assay components.

Conclusion. We have proposed a novel homogenous assay system which can be used for characterization of ligand binding to different GPCR and their complexes with G proteins. Positive results have been obtained in the case of receptors for peptides like melanocortin (MC4R) and neuropeptide Y (NPY1R) as well as for receptors of monoamines like dopamine (D1DAR) and serotonin (5-HT1AR).

Acknowledgements. This work has been financed by Estonian Ministry of Education and Science (IUT 20-17) and by the European Regional Development Fund (TK114, 30020).

L10

EPHAR SPONSORED LECTURE

TREATING THE BRAIN WITH DIABETES DRUGS IN ALZHEIMER'S DISEASE

Jørgen Rungby

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Epidemiology links type-2 diabetes with a number of neurodegenerative diseases, notably Alzheimer's disease (AD). A doubling or more of the relative risk accompanies type-2 diabetes for AD. A long search for common pathological features has identified several: Neuronal insulin resistance, amyloidosis, low-grade inflammation, and altered glucose metabolism. Further, cognition declines more rapidly in the elderly with diabetes and seems to be negatively affected even by pre-diabetic increases in glycaemia. This has led to optimism that targeting classical diabetes mechanisms may improve outcome in AD and MCI and the term type 3 diabetes has accordingly been coined for AD. Pre-clinical data - most gathered in rodents - suggest beneficial effects of metformin, insulin, thiazolidinediones, DPP-4 inhibitors/GLP-1 receptor agonists, and amylin. Clinical studies are conflicting. While no positive effects could be found with thiazolidinediones, intranasal insulin seems to hold some promise. GLP-1 receptor agonists, able to influence intracerebral glucose metabolism, are currently being tested in both exploratory and outcome studies, some of which will be presented.

L11

EPHAR SPONSORED LECTURE

IDENTIFICATION OF NOVEL GENES ASSOCIATED WITH ALZHEIMER'S DISEASE BY FUNCTIONAL GENOMIC ANALYSIS IN A HSV-1 INFECTION MODEL OF NEURODEGENERATION

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Alzheimer's disease (AD), the single most common cause of dementia, is characterized by massive neuronal damage leading to cerebral atrophy and the loss of cognitive function. Most AD cases (>95%) are sporadic. Sporadic AD is a highly complex disease for which neither the causal agent(s) nor the molecular mechanisms behind are well known. Among the environmental risk factors, persistent brain infections, particularly those induced by Herpes simplex virus type 1 (HSV-1), seem to play a key role in AD pathogenesis. Another factor is oxidative stress, intimately linked to aging and, therefore, thought to be crucial to the onset and development of the disease. Our group works with both factors to simulate the sporadic form of AD in vitro. The objective of the present study is the identification of genes associated with AD to provide novel diagnostic tools predicting the risk and/or the progression of the symptoms, and to identify possible therapeutic targets for the disease. Using gene expression studies of the human neuroblastoma cell line SK-N-MC, we have identified a set of oxidative stress-regulated genes in infected cells and in a cell model of familial AD (APPswe). Several data mining techniques, such as enrichment analysis, have been used for the interpretation of the experiment. It suggests that the interaction of oxidative stress with both HSV-1 and mutant APP alters lysosomal function. These data support earlier findings and those of other authors highlighting the role of lysosomes and of the two main pathways converging on it (autophagy and endocytosis) in early stages of AD neurodegeneration. To strengthen this hypothesis, ongoing work by our group is focused on the lysosomal pathway in our cell models. So far we found that HSV-1 infection leads to an increase of lysosomal content, decreased activity of several lysosomal cathepsins, and alterations in the degradation of endocytosis-mediated proteins. Taken together, these results indicate the endosomal-lysosomal pathway is altered. Additionally, the examination of the modulated gene list led to a selection of SNPs covering 5 candidate genes which are currently being tested in human samples for genetic association with AD. Out of these 5 candidates, the most promising ones will be used for their functional validation in our cell models, by modulating their expression (overexpression/knock-down) with posterior analysis of the neurodegenerative markers characteristic for AD.

L12

STUDIES OF THE BRICHOS DOMAIN, INSIGHTS INTO AN ANTI-AMYLOID CHAPERONE

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Background. Alzheimer's disease is an incurable neurodegenerative disorder linked to misfolding and aggregation of the amyloid β-peptide (Aβ). What causes Alzheimer >s disease is not fully understood, but it is believed that the transition of unstructured monomeric Aβ into β-sheet rich oligomers and fibers is a key element. Recent studies have revealed that once Ab42 fibrils are generated, their surfaces effectively catalyze the formation of neurotoxic oligomers. The BRICHOS domain is a ~100 residue domain found in membrane proteins. The name BRICHOS comes from Bri2, Chondromodulin-1, and Surfactant protein C (SP-C). Mutations of the BRICHOS containing proteins are associated with degenerative disease including lung fibrosis (proSP-C) and familial dementia (Bri2).

Methods. Recombinant protein production, Size exclusion chromatography Thioflavin T fluorescence, CD spectroscopy, NMR spectroscopy, x-ray crystallography, *Drosophila in vivo* model etc.

Results. *In vitro* experiments show that proSP-C and Bri2 BRICHOS can delay aggregation of Aβ, far below stoichiometric ratios. Thioflavin T fluorescence experiments to monitor Aβ42 aggregation kinetics reveal that when BRICHOS is present, the lag phase is prolonged and higher concentrations of Bri2 BRICHOS also decrease the elongation rate. By coating the Aβ fibrils, the BRICHOS domain can redirecting the aggregation reaction to a pathway that involves minimal formation of toxic oligomeric intermediates. This is verified with electrophysiology measurements and *in vivo* in *Drosophila Melanogaster*. Larger oligomers of Bri2 and Bri3 BRICHOS also possess general chaperon properties. We have also showed that BRICHOS can inhibit aggregation of other amyloidogenic proteins such as IAPP, Tau and prion fragments and α-synuclein.

Conclusions. We have investigated the BRICHOS domain from both proSP-C, Bri2 and Bri3 proteins. All three BRICHOS domains are able to inhibit fibril formation of Aβ and other aggregation prone peptides. The main mechanism of action is to block the secondary nucleation and therefore suppress the generation of toxic oligomeric species. This have been confirmed with ex vivo measurements on living mouse brain slices and in *Drosophila in vivo* measurements.

Acknowledgements. This work was supported by the Swedish Research Council, the Swedish Alzheimer Foundation, the Magnus Bergvalls foundation, Foundation of Gamla Tjänarinnor, the Loo and Hans Ostermans foundation. Henrik Biverstål is funded by the InnovaBalt project at the Latvian Institute of Organic Synthesis.

L13

MODIFICATIONS OF NO PRODUCTION IN BRAIN BY HALOGENATED VOLATILE ANESTHETICS AND NATURAL COMPOUNDS

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Background. Demand for both NO donors and NOS inhibitors in pharmaceutics is constantly growing. The expected activity of a novel drug is usually either predicted from its chemical structure, or conclusion about impact of a substance on NO production in the organism is made on the basis of in vitro experiments. Unforeseen effects are observed from time to time.

Methods. NO production was determined in rat brain tissues by means of ESR using Fe (DETC) as a spin trap.

Results. It is generally accepted that HVA inhibit nitric oxide synthase (NOS) activity and suppress the NO neurotransmitter function. However we have detected strong increases in NO concentrations in rat brain cortex following **halothane**, **sevoflurane** and **isoflurane** anesthesia, however **sevoflurane** and **isoflurane** caused NO decrease in cerebellum. These changes were abolished by the iNOS inhibitor AMT. The drastic increase in NO concentration in brain cortex after intraventricular LPS administration was enhanced by anesthesia with **isoflurane** and **sevoflurane**. **Isoflurane** was found to inhibit recombinant nNOS and iNOS activities in vitro at high concentrations only whereas **sevoflurane** was an even less potent inhibitor of the purified enzymes. Taken together our data suggest a putative role for iNOS in the increase in NO levels produced by halogenated volatile anesthetics whereas nNOS activity is probably inhibited during the anesthesia. Quercetin despite its iNOS-inhibiting activity observed in vitro by others decreased NO production in cerebellum of the LPS-treated animals and increased NO production in cerebellum of healthy animals. We observed enhancing effect on the LPS-induced NO production in brain cortex by **ellagic** acid, however NO production in cerebellum of the LPS-treated animals was decreased. **Resveratrol** is known to stimulate eNOS and to inhibit iNOS. Amazingly we observed only increase of NO production in brain cortex. The stilbene also enhanced the LPS effects in brain cortex and cerebellum. **Indole carbinol** decreased expression of iNOS gene in brain cortex and decreased the radical concentration in cerebellum of both intact and LPS-treated animals. The drug enhanced the LPS-induced outburst of NO synthesis in brain cortex. We have detected decrease of iNOS expression in brain cortex of **luteolin**- and **lycopene**-treated animals, however this inhibition did not produce any impact on NO production.

Conclusions. Taken together our results suggest that modifications of NO level in tissues by a compound cannot be predicted from data about its effects on NOS expression or activity studied in model systems.

Our data indicate special importance of direct measurements of NO concentration in the studies of the drug mechanism of action.

L14

HUMAN DENTAL PULP STEM CELLS AS PROMISING TOOLS FOR NEUROREGENERATION

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Background. Recent findings demonstrated the importance of paracrine mechanisms in the therapeutic action of human mesenchymal stromal cells (MSCs). Several studies demonstrated that in contrast to the MSCs derived from other tissues, MSC-like cells isolated from dental pulp (also known, as stem cells derived from the dental pulp of human exfoliated deciduous teeth, SHEDs) have unique neurogenic properties which could be potentially exploited for therapeutic use. We recently demonstrated that exosomes derived from SHEDs suppress 6-hydroxy-dopamine-induced apoptosis in human dopaminergic neurons. Importantly, we found that therapeutic properties of exosomes depended on culture conditions of SHEDs. Therefore, we investigated, how 3D microcarrier cell culture affects proteomic and miRNA signatures of exosomes derived from SHEDs.

Methods. Exosomes were purified by ultracentrifugation from SHEDs cultivated under two conditions: standart two-dimensional culture flasks, or from SHEDs grown on the laminin-coated microcarriers in bioreactor (BioLevitator™, Hamilton). In both cases cells were grown in serum- and xeno- free medium (MSC NutriStem XF, Biological Industries). For proteomic studies liquid chromatography coupled to tandem mass spectrometry analysis was carried out on an EASY-nLC connected to a Velos Pro-Orbitrap Elite hybrid mass spectrometer with nano electrospray ion source (Thermo Fisher Scientific). For miRNA profiling we used the human miRNome miScript miRNA PCR Array (Qiagen).

Results. In total we identified 80 proteins in exosomes from standart SHED cultures and 60 proteins in exosomes from microcarrier cultures. The majority of the identified proteins are included in the vesiclepedia database. Importantly, only 28 proteins were common between exosomes from different preparations.

Profiling of the expression of the 1008 most abundantly expressed and best characterized miRNA sequences in the human miRNA genome revealed significant differences between exosomes derived from SHEDs grown under different conditions. We found, that 3D exosomes display richer repertoire of miRNAs-32 % of positive reactions from all miRNAs tested versus 24 % of positive reactions in exosomes from standart SHED cultures. We also detected significant increase of expression (by up to 52-67 folds) of some of miRNAs (hsa-miR-199b-5p, hsa-miR-31-5p and hsa-miR-4291 miRNR) in 3D exosomes. By contrast, exosomes from standart SHED cultures uniquely expressed hsa-miR-188b-5p.

Conclusions. Our findings indicate, that 3D microcarrier cell culture have a profound impact on the proteomic and miRNA signatures and, possibly, physiological properties of exosomes. These studies will help to identify neuroprotective factors of SHED exosomes.

Acknowledgements. This research was funded by the European Social Fund under the Global Grant measure (No. VP1-3.1-ŠMM-07-K-03-016).

L15

EXOSOMES FROM DENTAL PULP STEM CELLS RESCUE HUMAN DOPAMINERGIC NEURONS FROM 6-HYDROXIDOPAMINE INDUCED APOPTOSIS

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Background. Human mesenchymal stromal cells (MSC) are widely used in fundamental and clinical studies. However, in some instances, paracrine factors secreted by MSCs may have better therapeutic effects than the cells by themselves. Here we present new data about the effects of exosomes derived from MSC-like cells isolated from dental pulp (also known, as stem cells derived from the dental pulp of human exfoliated deciduous teeth, SHEDs) on 6-hydroxydopamine (6-OHDA)-induced apoptosis of human dopaminergic neurons.

Methods. ReNcell VM human neural stem cells (Millipore) were differentiated into dopaminergic neurons and treated with 100 µM 6-OHDA alone, or in combination with exosomes purified by ultracentrifugation from SHEDs cultivated under two conditions: standart two-dimensional culture flasks, or from SHEDs grown on the laminin-coated microcarriers in bioreactor (BioLevitator, Hamilton). Real-time monitoring of apoptosis was performed using Leica SP8 (Leica Microsystems) confocal microscope and CellEvent™ Caspase-3/7 green detection reagent (Life technologies).

Results. 6-OHDA effectively induced apoptosis in human dopaminergic neurons. Exosomes derived from SHEDs cultured under standart conditions enhanced cell apoptosis. Microvesicles derived from dental pulp stem cells had no marked effect on apoptosis. Exosomes derived from SHEDs cultured in bioreactor significantly suppressed apoptosis of human dopaminergic neurons.

Conclusions. Exosomes from SHEDs grown on the laminin-coated three-dimensional alginate microcarriers significantly suppressed 6-OHDA-induced apoptosis in dopaminergic neurons. By contrast, exosomes from standart SHED cultures acted as sensitizers to 6-OHDA in neuronal cultures. These findings indicate that culture conditions profoundly affect anti-apoptotic properties of SHED exosomes.

Our results demonstrate that exosomes derived from SHEDs are potential new therapeutic tools against Parkinson's Disease

Acknowledgements. This research was funded by the European Social Fund under the Global Grant measure (No. VP1-3.1-ŠMM-07-K-03-016).

L16

NEUROGLIAL PROPERTIES OF SKIN-DERIVED MESENCHYMAL STEM CELLS AND DEVELOPMENT OF IN VITRO MODEL FOR DRUG TESTING

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Background. Skin-derived mesenchymal stem cells (S-MSCs) are multipotent stem cells that have the capacity to differentiate into mesodermal and neuroectodermal cell lineages. It is proposed that S-MSCs originate from neural crest stem cells and they could act as the modulators of the peripheral neural stem cell niche. The niche modulating effect could be reached by paracrine effect, changes into the extracellular matrix composition or changes in the cell phenotype. The aim of the current study was to establish *in vitro* S-MSC neirodifferentiation model that could be used to find compounds with neuroregeneration promoting effect.

Methods. S-MSCs were propagated in neural progenitor induction medium containing retinoic acid (RA). Afterwards, medium containing NT-3 was added to induce neuronal differentiation. For S-MSC glial differentiation the Schwann precursor cell medium containing neuregulin1β and forskolin was used. Cells were analyzed for neuronal marker tubulin βIII, p75NTR, integrin α6 (ITGA6), integrin β1 (ITGB1), glial marker S100B, SOX10 and neural crest marker integrin α4 (ITGA4), Notch1, Ap2α, Pax6 expression by qPCR method. Nestin, GFAP, tubulin βIII, SOX10 and CD271 expression was analyzed by immunofluorescence (IF) and flow cytometry (FC). Additionally, sigma-1R expression was studied by qPCR and IF. The study on human cells was approved by the University of Latvia, Institute of Clinical and Experimental Medicine ethical committee.

Results. qPCR analysis revealed 7-fold increase of *p75NTR* and *ITGA4*, 3-fold increase of *ITGA6* and 2-fold increase of *ITGB1* expression in S-MSC (n=3) propagated in neural progenitor induction medium. Increased p75NTR expression during neural differentiation was confirmed by FC analysis. We observed 2-fold increase of *S100B* expression in S-MSCs cultivated in Schwann cell precursor medium. Interestingly, sigma-1 receptor expression was detected by IF analysis in S-MSCs propagated in Schwann cell precursor medium. However, qPCR data revealed 3-fold increase of sigma-1R in RA containing neural progenitor induction medium.

Conclusions. S-MSCs expressed markers p75NTR, ITGA4, ITGA6 and ITGB1 when propagated in the neurodifferentiation medium. Glial marker S100B and sigma-1R were expressed in S-MSCs during Schwann cell differentiation. Altogether our data suggest that the current S-MSCs neurodifferentiation protocol could be used as *in vitro* model to test the ability of various compounds to influence S-MSC differentiation towards Schwann or neural cell phenotype.

Acknowledgements. The study was supported by Taiwan-Lithuania-Latvia joint research grant "Mesenchymal stem cell and cancer stem-like cell response to nanoparticle treatment".

POSTER ABSTRACTS

P1

FLUORESCENCE ANISOTROPY BASED ASSAY FOR CHARACTERIZATION OF LIGAND BINDING TO DOPAMINE RECEPTORS

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Background. Dopaminergic receptors are G-protein-coupled receptors (GPCRs), which are involved in a wide variety of physiological processes. Abnormalities in GPCR mediated signal transduction are associated with many different diseases. Therefore, dopamine receptors are targets for variety of drugs involved in diseases like schizophrenia, Parkinson's disease, depression and many others. In order to develop drugs with less side effects and better efficacy it is necessary to understand and characterize receptor-ligand interactions in further details.

Methods. We have applied fluorescence anisotropy (FA) assay to investigate kinetic properties and affinities of different ligands for dopamine D1 receptor. For that we have implemented budded baculoviruses as a source of recombinant protein (Veiksina et al., 2014).

Results. We have seen that fluorescent ligand Bodipy-FL-SKF-83566 is suitable for the pharmacological characterization of non-labelled dopaminergic ligands. The obtained results are in general agreement with the data obtained from radioligand [3H]SCH 23390 binding experiments with the same baculovirus preparations. Furthermore, we are now able to perform real-time monitoring of ligand binding.

Conclusion. Our results show that fluorescence anisotropy based assay is applicable for the study of dopamine receptors and their ligands.

Acknowledgements. We thank Dr. Stephen Briddon from the University of Nottingham (UK) for providing us Bodipy-FL-SKF-83566. This work has been financed by Estonian Ministry of Education and Science (IUT 20-17) and by the European Regional Development Fund (TK114, 30020).

P2

KINETIC CHARACTERIZATION OF MELANOCORTIN-4 RECEPTOR LIGAND BINDING USING FLUORESCENCE ANISOTROPY

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Background. Melanocortin receptors (MCRs) that belong to the superfamily of G protein-coupled receptors are known for their broad physiological relevance. Melanocortin-4 (MC4) receptors are one of the most functionally important subtypes as they are responsible for energy homeostasis, eating behavior and regulation of sexual functions, and therefor are an important drug target (Wikberg et al., 2008). MCRs are governed by a complex dynamic homotropic regulation, which requires a high-quality assay for analyzing complex receptor-ligand interactions (Kopanchuk et al., 2006). There is an increasing trend towards using fluorescence anisotropy (FA) for analyzing receptor-ligand interactions. FA allows continuous monitoring of ligand binding processes and characterization of ligand binding dynamics (Veiksina et al., 2010). Exposing the receptors of interest on the surface of budded baculovirus particles greatly increases the quality of the assay (Veiksina et al., 2014). The homogeneous budded baculovirus/FA-based assay system combined with a selection of reporter ligands could become a valuable tool for ligand screening.

Methods. The use of a fluorescent ligand Cy3B-NDP-α-MSH has made it possible to study MC4 receptors with higher precision and sampling rate (Veiksina et al., 2014). However this ligand has relatively slow kinetics. Modification of the structure of a MC_4 receptor antagonist revealed two new reporter ligands (UTBC101 and UTBC102) for fluorescence labeling. These new reporter ligands selectively bind to MC4 receptors and exhibit improved kinetic properties. Quantitative multivariable global analysis was used to determine the kinetic parameters of the new ligands (Veiksina et al., 2014).

Results. Compared to Cy3B-NDP-α-MSH, UTBC101 has higher association and dissociation rate constants, k_{on} = ((2.0 ± 0.6) x 107 M^{-1} min^{-1}) and koff = ((4.6 ± 0.3) x 10^{-3} min^{-1}). UTBC102 has a similar association rate constant to UTBC101, kon = ((2.1 ± 0.7) x 107 M^{-1} min^{-1}), but an even higher dissociation rate constant, k_{off} = ((1.3 ± 0.3) x10^{-1} min^{-1}).

Conclusions. Both new ligands, UTBC101 and UTBC102, have been found to be suitable for continuous monitoring of binding reactions using the budded baculovirus/FA-based assay, thus enabling characterization of both labelled and non-labelled ligand binding dynamics in regard to the MC4 receptor. Due to its very high dissociation rate, which enables to achieve equilibrium conditions, UTBC102 could be especially valuable for screening pharmacologically active compounds.

Acknowledgements. This work was financed by the Estonian Ministry of Education and Science (IUT20-17) and by the European Union through the European Regional Development Fund (TK114, 30020).

P3

FLUORESCENCE ANISOTROPY BASED ASSAY FOR CHARACTERIZATION OF LIGAND BINDING TO DOPAMINE RECEPTORS

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Background. Dopaminergic receptors are G-protein-coupled receptors (GPCRs), which are involved in a wide variety of physiological processes. Abnormalities in GPCR mediated signal transduction are associated with many different diseases. Therefore, dopamine receptors are targets for variety of drugs involved in diseases like schizophrenia, Parkinson's disease, depression and many others. In order to develop drugs with less side effects and better efficacy it is necessary to understand and characterize receptor-ligand interactions in further details.

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P4

ENHANCING OSTEOGENIC DIFFERENTIATION OF HUMAN PERIODONTAL LIGAMENT STEM CELLS BY USING 3D MICROCARRIER CELL CULTURE

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Background. Regeneration of periodontal tissue represents major chalenge of modern tissue engineering. In this study, we adapted novel microcarrier technology for the cultivation and osteogenic differentiation of human periodontal ligament stem cells (PDLSC).

Methods. Primary cell lines of PDLSCs were isolated from human periodontal ligament of extracted intact premolars for orthodontic reasons. Material was collected under the approval of the Lithuanian Bioethics committee. PDLSC lines were isolated using explantation method and cultured under standart conditions (LG-DMEM plus 10 % FBS) until passage 4th, then PDLSCs were transferred on gelatin-coated flasks (2D), or on the gelatin-coated microcarriers (3D) and cultivated in bioreactor (BioLevitator™,Hamilton).

Osteogenic differentiation was induced by 0,1 µM dexamethasone, 50 µg/ml ascorbic acid and 10 mM β-glycerophosphate. Detection of extracellular calcium deposits has been performed by staining with Alizarin RED S at 14 and 21 days after induction of differentiation.

Total RNA was isolated from control and differentiating (at 14 and 21 day) 2 D and 3D PDLSC cultures. Real-time polymerase chain reaction was performed using the CFX96 instrument (Biorad) and CFX manager software (version 3.1). The expression levels of alkaline phosphatase (ALP) and osteopontin (OPN) were analyzed using Maxima SYBR Green qPCR/ROX Master mix (Fermentas).

Results. Alizarin staining revealed no calcium deposits in control and differentiating 2 D PDLSC cultures. By contrast, differentiating 3D microcarrier cell cultures exhibited a dramatic increase in mineralization. qPCR analysis revealed high expression levels of ALP and OPN genes in control 3D PDLSC cultures when compared to 2D PDLSC controls. We also observed gradual decrease of ALP and OPN gene expression during osteogenic differentiation of PDLSCs grown under both conditions.

Conclusions. We demonstrate, that 3D microcarrier culture increase osteogenic differentiation capacity of PDLSCs.

P5

DUAL MODALITY FLUORESCENCE AND COMPUTED TOMOGRAPHY SYSTEM FOR SMALL ANIMAL IN VIVO IMAGING

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Background. True 360° fluorescence tomography system that captures light signals all around animal could ensure realization of full potential of in vivo optical imaging. To find the exact fluorophore location in the animal, computed tomography (CT) is used for anatomical reference and images from two systems are combined into one that could result in sub-optimal results due to differences in animal positioning in each of the imagers. By combining X-ray and fluorescence emission tomography into one system it is possible to acquire multimodal images and analyze data with minimized risk of reposition errors. When fluorescent probes are used for small animal in vivo optical imaging, the best results are achieved when near-infrared (NIR) fluorescence emitting fluorophores are used. NIR agents with emission wavelengths between 700-900 nm are optimal for in vivo applications due to their low tissue auto-fluorescence and good tissue penetration. InSyTe FLECT/CT true 360° tomography system (Trifoil Imaging) is the first imager that combines CT with true 360° fluorescence emission tomography (FLECT). In the present study, we applied Trifoil Imaging system to assess its potential use in animal models of neuro-inflammation.

Methods. Nude SKH1 male mice (25-28 g) were monitored for up to 2 days after injections of fluorescent probes. *NiraWave* nano 780 probe was used to monitor various fluorophore concentrations after subcutaneous injections of different quantities of probe. Inflammation-activatable *ProSense 750EX* probe was used to detect the progression of neuro-inflammation in the mice brain after intracisternal administration of lipopolysaccharide (LPS). For excitation of probes, a 705 nm laser with corresponding filters (803 nm (wide) for *NiraWave nano 780* probe and 813 nm (narrow) for P*roSense 750EX* probe) were used. FLECT images were reconstructed using maximum available 116 source points for each slice of 1 mm thickness. Image analysis was performed using VivoQuant software.

Results. Imager was able to adequately acquire and reconstruct images of various fluorophore concentrations after subcutaneous injections of *NiraWave* nano 780 probe. Reconstructed images were suitable for further data analysis. Using *ProSense 750EX* probe, the LPS-induced brain inflammation was detected and precise location and time-dependent changes in size of inflamed area were determined.

Conclusions. Using a true 360° fluorescence emission tomography system and appropriate fluorescent probes allows accurate imaging of the progression of neuro-inflammation.

P6

INTEGRATIVE APPROACH TO THE TREATMENT OF DIABETIC FOOT SYNDROME CAUSED BY DIABETIC SENSORYMOTOR NEUROPATHY: A CASE STUDY

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Background. Over half of diabetic patients are developing symptoms of sensory neuropathy caused by several molecular mechanisms, including accumulation of AGEs, activation of polyol pathway, protein kinase C, inflamation, etc. One of the most serious complications of diabetic peripheral neuropathy is the diabetic foot syndrom. There is no unified concept of treatment of diabetic neuropathy, different drugs can be applied e.g. antioxidants (alpha-lipoic acid). However, in many cases results from conventional therapy might not provide expected outcomes hence integrative approch combining a modern biomedicine with a complimentary whole system medicine like Ayurveda can provide better outcomes clinically and improved quality of life.

Patient information. Sixty six years old nonobese male with no known family history of Type two Diabetes (T2D) presented with complains of multiple nonhealing ulcers in the right foot, the largest lesion involving the first toe and the plantar surface below it. The associated symptoms were extreme pain, burning, tingling, low grade fever and foul smelling pale yellow pus discharge. One year back patient had developed similar complains in the second toe of the same foot because of which he approched a general physician and confirm to have T2D. After intensive per-oral hypoglycemic therapy and wound management his glycemic index improved but ulceration continued for which he had to amputate his second toe. Due to the reoccurence of symptoms in his first toe after six mounts patient visited outpatient department of AVCRI outpatient clinic, at that point he was on pioglitazone and repaglinide. After Ayurvedic clinical examination and diagnosis the therapy was implamented involving internal medication including herbs such as *Curcuma longa*, *Tinospora cordifolia*, *Rubia cordifolia, Cassia fistula, Azadirachta indica, Strychnos potatorum, Rubia cordifolia, Pichoriza kurroa, Raphanus sativus* proven to have hypoglycemic, antiinflamatory, antimycrobial, antioxidant, wound healing, angiogenetic and vasodilatative effects. Daily external treatment of the wounds was done by washing and herbal fumigation using *Curcuma longa, Emblica officinalis, Terminalia bellirica, Terminalia chebula, Cassia fistula and Commiphora wightii*. Within two mounts of active treatment all the above symtoms were resolved, healthy granulation achieved and epithelization resumed.

Conclusions. This case gives us an insight into succesful integration of Ayurvedic intervention along with conventional antidiabetic therapy in the treatment of neurological and vascular T2D complications leading to diabetic foot syndrome.

P7

EVALUATION OF RGB IMAGING SYSTEM FOR IN VIVO ASSESMENT OF CUTANEOUS VASOREACTIVITY AND MICROCIRCULATION

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Background. Recently there is a growing evidence for the association of central and peripheral nervous system pathologies with the skin diffuse neuroendocrine system (SDNES) function. The relation has been shown for neurodegenerative disease such as Parkinson, Alzheimer's disease (Rodríguez-Leyva et al., 2014), and fibromyalgia (Albrecht et al., 2013).Therefore the promising diagnostic strategies for the large-scale population screening could be in vivo evaluation of cutaneous vasoreactivity and microcirculation. However presently available test procedures require expensive equipment and experienced operators limiting its utilization. The aim of present study was to develop and evaluate cost-effective RGB imaging system for assessment of cutaneous vasoreactivity and microcirculation.

Methods. To assess cutaneous microcirculation the custom made RGB imaging system prototype (RGBi) has been developed. It comprised uniform white light source (24 white LEDs), color CMOS camera (USB uEye LE form IDS Imaging Development Systems GmbH) and portable computer. Speckle reduction has been achieved implementing orthogonal polarization technique. Twelve healthy volunteers (2 males and 10 females) were enrolled, and evaluation of RGBi system has been performed comparing the RGB data obtained during skin local heating (dorsal aspect of the palm, for 28 min 20-44°C) to that acquired by laser Doppler imager (LDPI system-moorLDI2 from Moor Instruments, UK).The recorded RGB video was processed by selecting the region of interest (RoI) of the heat exposed skin area and mean intensity values extracted for each color channel. The RGBi perfusion was estimated as ratio of the red and green channel direct component (dc) signal and attributed to the total blood volume changes.

Results. Both RGBi and LDPI demonstrated same trend which is typical for local heating test: neuropeptide induced initial transient vasodilatation peak lasting for 6-8 minutes with the following prolong nitric oxide evoked vasodilatation phase. There was a statistically significant correlation ($P<0.05$; $r=0.94$) between mean perfusion index obtained by LDPI and RGBi.

Conclusions. The custom made RGB imaging system has a great potential as cost-effective alternative to LDPI utilized in large population scale screening of SDNES function. However for clinical use further more extensive evaluation is required.

P8

THE EXTRACELLULAR MATRIX MOLECULE TENASCIN-R AFFECTS THE NEURONAL EXPRESSION OF PROLYL ENDOPEPTIDASE

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Background. Extracellular matrix (ECM) molecules are expressed in specialized neurons and glial cells at the cell soma or axonal surfaces and play a pivotal role in cellular communication and during normal brain pattern formation, but also in the course of diverse neuropathologies and tissue repair. One of these ECM molecules is the glycoprotein tenascin-R (tn-R) which is exclusively expressed in the brain of vertebrates by neurons and oligodendrocytes. Tn-R has been implicated in a variety of functions, e.g. during myelination, cerebellar neurite fasciculation and hippocampal long-term potentiation (LTP). In the hippocampus, tn-R is located between fasciculating nonmyelinated axon bundles of mossy fibers and axons of the perforant path, between cell somata of CA3 pyramidal neurons and at gap junctions between astrocytic processes. In addition, decreased perisomatic inhibition, increased basal excitatory synaptic transmission and reduced LTP were observed in the hippocampal CA1 region of tn-R knock-out mice. In this study, we focused on the affect of tn-R deficiency onto the prolyl endopeptidase (PREP), a serine protease, involved in cell division, signal transduction, learning and memory. Further, neuronal cell adhesion molecule (NCAM), matrix metalloprotease 9 (MMP9) and tissue inhibitor of MMPs 1 (TIMP-1) was investigated.

Methods. To determine the expression of PREP, MMP9, TIMP-1, NCAM and psa-NCAM, in tn-R deficient and wild type mice brains, western blot and immunohistochemistry were carried out.

Results. Immunohistochemical and western blot analyses of hippocampus tissues revealed a significant reduction of PREP and an increase of MMP9 in tn-R knock-out mice compared to wild type mice. Also, it seems that PREP and MMP9 are co-localized in the same cells. In addition, the specific MMP9-inhibitor TIMP-1 and NCAM are increased in the hippocampus of tn-R knock-out mice.

Conclusions. MMP9 can protolytically cleave tn-R to smaller fragments which in turn could be potential substrates for PREP. Additionally, MMP9 plays a critical role in inflammatory processes. These results indicate that the tn-R deficient mice are more vulnerable to inflammation processes as well, which might have an affect on brain plasticity and LTP.

Acknowledgements. This research was financed by the European social fund (ESF).

P9

REGULATION OF NO· BIOSYNTHESIS BY NATURAL COMPOUNDS IN CEREBELLUM AND CORTEX

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Background. Natural biologically active compounds of plant origin including flavonoids are widely used as dietary supplements or drugs. Many plant derived compounds manifest antioxidant, anti-inflammatory, anti-oestrogenic, anti-mutagenic and anticarcinogenic effects. However, biological activity of polyphenol-rich food products does not correlate with effects that could be deduced from effects of individual compounds on NO· synthase activity. We hypothesize that effects on NO· production of flavonoids and other natural compounds is pleiotropic and that affect NO· production at different steps: NOS gene expression, stability of mRNAs, synthesis of NOS proteins and their maintenance in the cells, activity of enzymes and stability of produced radicals.

Methods. The analyzed natural compounds were resveratrol, caffeic acid phenethyl ester, quercetin, kaempferol, luteolin, myricetin, baicalein, ellagic acid, indole-3-carbinol and lycopene at 50mg/kg concentration. The sepsis model in rats was induced by i/p LPS injection and after four hours the tissues were taken for analysis. The NO· radical concentrations in rats brain cortex and cerebellum were detected by electron paramagnetic resonance. RT-qPCR and immunohistochemical examinations were used to measure iNOS mRNA and protein levels.

Results. Resveratrol increases NO· production in rats cerebellum by 85% ($p<0,001$), but caffeic acid phenethyl ester decrease by 29% ($p<0,05$) in condition of LPS induced sepsis. There were several natural compounds who increase NO· level in LPS stimulated rats brain cortex - resveratrol by 107% ($p<0,001$), ellagic acid by 40% ($p<0,001$), luteolin by 21% ($p<0,05$), indole-3-carbinol by 25% ($p<0,001$), while caffeic acid phenethyl ester decreases NO· production by 21% ($p<0,01$). Evaluating natural compounds influence on iNOS protein expression in rats brain cortex under condition of sepsis, none of them showed statistically significant changes, while indole-3-carbinol increased the expression of iNOS mRNA in the cortex 3.5 fold ($p<0,01$) in sepsis.

Conclusions. Caffeic acid phenethyl ester decreased NO· level under sepsis condition in brain cortex and cerebellum and can be regarded as a potential inflammation-reducing substance in the brain tissue.

P10

SUBCUTANEOUS AND INTRAPERITONEAL MK-801 ADMINISTRATION LEADS TO DIFFERENT BEHAVIOURAL OUTCOMES IN MORRIS WATER MAZE TEST IN RATS

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Background. Dizocilpine, also known as MK-801, is one of the most popular NMDA receptor antagonists used experimentally to induce the impairment of memory and learning. In previous experiments, different MK-801 doses and routes of administration have been used and resulted in conflicting data. The aim of the present study was to evaluate effects of different doses and administration routes of MK-801 on behaviour outcomes in the Morris water maze test.

Methods. MK-801 was injected either intraperitoneally (*i.p.*) or subcutaneously (*s.c.*) at doses of 0.01, 0.05 and 0.1 mg/kg in *Wistar* rats 30 minutes before the first training trial. The rats were trained in the pool for 5 consecutive days, 4 trials/day. Rats were allowed to swim until they found the platform or 90 seconds elapsed. As a measure of learning process, the escape latency was recorded as the time until rat reached the platform in each trial. The retention latency represented the time spend in the platform sector and it characterized memory processes. The retention latency was tested 24 h and 72 h after the last training session. The concentration of MK-801 was measured in brain tissue and plasma.

Results. MK-801 administered *s.c.* at doses of 0.1 and 0.05 mg/kg significantly increased the escape latency compared to *i.p*. administration at the same dosage. MK-801 induced motor disturbances at the 0.1 mg/kg dose after s.c administration. All MK-801 groups showed significant impairment of memory at 24 h and 72 h after *i.p.* and *s.c* administration. MK-801 administered *s.c.* at doses of 0.1 and 0.05 significantly impaired memory compared to the same doses of *i.p.* administrations after 24 h. MK-801 concentration in brain tissues and blood plasma samples was 1.5 fold higher after *s.c.* than *i.p.* administration at the dose of 0.1 mg/kg.

Conclusions. Our data suggest that s.c. administration of MK-801 leads to higher brain tissue concentration and more impaired spatial learning and memory compared to *i.p.* administration. The optimal dose for memory impairment effects of MK-801 in case of *s.c.* administration is 0.05 mg/kg.

Acknowledgements. This research was financed by the grant from the Latvian Council of Science (108/2013).

P11

CHANGES OF COMMON FEMORAL ARTERY HEMODYNAMIC PARAMETERS DURING UNILATERAL POST-OCCLUSIVE REACTIVE HYPEREMIA

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Background. Recent literature suggests the role of sympathetic nervous system (SNS) in modulation of conduit artery local hemodynamic factors (linear velocity and shear rate) and their effect on endothelial function. Thus the activation of the SNS leads to an immediate increase in retrograde shear stress, which is associated with decreased endothelial function and in long term, might facilitate cardiovascular disease (Padilla et al., 2010). Presently, there is no generally accepted explanation on origin of blood flow linear velocity components and their influencing factors. Particularly intriguing phenomenon are artery blood flow velocity waveform changes in contralateral limbs during unilateral regional hemodynamic maneuvers when transient alterations of systemic cardiovascular parameters and sympathetic activation are induced.

The aim of this study was to evaluate alterations of common femoral artery (CFA) linear velocity waveform in contralateral leg during unilateral post-occlusive reactive hyperemia (PORH).

Methods. Study comprised 16 young and healthy women. Unilateral thigh arterial occlusion (200 mmHg) at different durations (3, 7 and 15 min) has been accomplished. During rest and PORH periods CFA flow linear velocity waveform and diameter traces were registered bilaterally using ultrasound Doppler in parallel to beat-to-beat recording of mean arterial pressure (MAP), heartrate (HR), cardiac output (CO) and total peripheral resistance (TPR) using Finapres device.

Results. During resting conditions there were no significant bilateral differences for CFA hemodynamic parameters. Hence in occlusion, linear velocity (TAM) and blood flow (Q) were substantially diminished only in occluded extremity. During PORH occluded leg CFA exhibited increase of Q (395 – 585% from baseline), increase of TAM (300 – 425%) and absence of retrograde flow, while in contrary-contralateral leg showed increased retrograde flow (175 – 325%) and decrease of Q (35 – 58%). Moreover PORH induced significant alterations of systemic cardiovascular parameters: increased HR (19 – 24%), CO (24 – 31%) and decreased TPR (22 – 26%) and MAP (7 – 12%).

Conclusions. Unilaterally applied PORH induced following alterations of flow velocity profile in contralateral leg CFA: a small decrease of anterograde component and significant increase of retrograde component. An increase of retrograde component was proportional to duration of occlusion and increased HR indirectly indicating the role of sympathetic vasoconstrictor response in contralateral leg.

P12

THE EFFECTS OF R-PHENIBUT IN EXPERIMENTAL MODEL OF ISCHEMIC STROKE

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Background. Ischemic stroke is one of the major causes of death and disability in adults worldwide. Motor impairment, sensory loss and cognitive deficits lead to immense reduction in the quality of life for stroke patients. R-Phenibut is a CNS active drug and γ-aminobutyric acid (GABA)-B receptor agonist. Previously it has been shown that GABA-B receptor agonists possess a neuroprotective effects in *in vitro* and *in vivo* models of ischemia. The aim of the present study was to test the effects of R-phenibut on the recovery of motor, sensory and tactile function and histological outcome in rats following transient middle cerebral artery occlusion (MCAO).

Methods. Transient MCAO for 120 min with silicon coated filament was used to model ischemic brain damage in male *Wistar* rats. Two hours after reperfusion animals received either R-phenibut at a dose of 10 mg/kg or 50 mg/kg or saline. The effects of R-phenibut on the motor, sensory and tactile functions were studied in the vibrissae-evoked forelimb-placing, limb-placing, beam-walking and cylinder tests at baseline and on post-stroke days 3, 7 and 14. The infarct size was quantified 14 days after MCAO using Image-Pro Plus 6.3. Quantitative real time-PCR was used to detect lipopolysaccharide-induced IL-1β, TNF-α and iNOS gene expression in male ICR mice after acute treatment with R-phenibut. The concentration of R-phenibut in brain tissue extracts and plasma was measured by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS). The statistical calculations were performed using the GraphPad Prism 3.0 software package.

Results. R-phenibut administration at a dose of 50 mg/kg significantly improved sensorimotor function in the cylinder test. R-phenibut inhibited lipopolysaccharide-induced iNOS gene overexpression in mice brain tissues. Behavioural improvement after R-phenibut treatment at a dose of 50 mg/kg significantly correlated with histological outcome.

Conclusions. The obtained results provide evidence for the neuroprotective activity of R-phenibut in an experimental model of stroke. These effects might be related to both the modulatory effects of the drug on the GABA-B receptor and the anti-inflammatory activities of the drug.

Acknowledgements. European Regional Development Fund project No. 2DP/2.1.1.1.0/13/APIA/VIAA/001 "Development of synthesis methods and investigation of pharmacological profile of active pharmaceutical ingredients".

P13

R-PHENIBUT EXERTS GABAPENTIN-LIKE ACTIVITY VIA THE A2-Δ SUBUNIT OF VOLTAGE-DEPENDENT CALCIUM CHANNELS

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Background. R-Phenibut ((3R)-phenyl-4-aminobutyric acid) is the optically pure and pharmacologically active form of racemic phenibut, a derivative of γ-aminobutyric acid (GABA). Phenibut is a clinically used anxiolytic, mood elevator and nootropic drug. The pharmacological activity of R-phenibut correlates to its binding affinity to GABAB receptors. Structurally, R-phenibut is related to baclofen and gabapentin (GBP), drugs that both mimic the chemical structure of the neurotransmitter GABA. Baclofen is a GABAB receptor-active compound, but GBP does not bind to these receptors and exerts its anti-nociceptive and anti-convulsant activity through binding to the α2-δ subunit of the voltage-dependent calcium channel (VDCC).

Methods. In this study, we tested the binding affinity of R-phenibut to the α2-δ subunit of the VDCC by using a subunit-selective ligand, radiolabelled GBP. In addition, we tested the anti-nociceptive and anti-seizure effects of R-phenibut in formalin-induced paw licking and pentylenetetrazole (PTZ)-induced seizure tests, respectively.

Results. The binding experiments revealed that the affinity constants for R-phenibut, baclofen and GBP in a rat brain membrane preparation were 26, 156 and 0.05 μM, respectively. In the PTZ-induced seizure test, we found that R-phenibut did not affect PTZ-induced clonic and tonic seizures at a dose of 50 mg/kg. Pre-treatment with R-phenibut decreased the nociceptive response in both phases of the formalin-induced paw-licking test in a dose-related manner in mice. The anti-nociceptive activity of R-phenibut was blocked by a GABAB receptor-selective antagonist, 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348), but this only occurred during the first phase of the test.

Conclusions. Our data suggest that R-phenibut binds to the α2-δ subunit of the VDCC with 4-times higher affinity than to the GABAB receptor. The anti-nociceptive activity of R-phenibut in the second phase of the formalin-induced paw-licking test is associated with its effect on the α2-δ subunit of VDCCs rather than through activity at GABAB receptors. In conclusion, our results provide experimental evidence for GBP-like anti-nociceptive properties of R-phenibut, which might be used clinically in treatment of neuropathic pain disorders.

Acknowledgements. This study was supported by the European Regional Development Fund, Project No. 2013/0030/2DP/2.1.1.1.0/13/APIA/VIAA/001.

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