INTERNATIONAL CONFERENCE OF THE BULGARIAN SOCIETY OF PHYSIOLOGICAL SCIENCES

Stara Zagora Mineral Springs, Bulgaria
October 30th - November 1st, 2022

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BOOK OF ABSTRACTS

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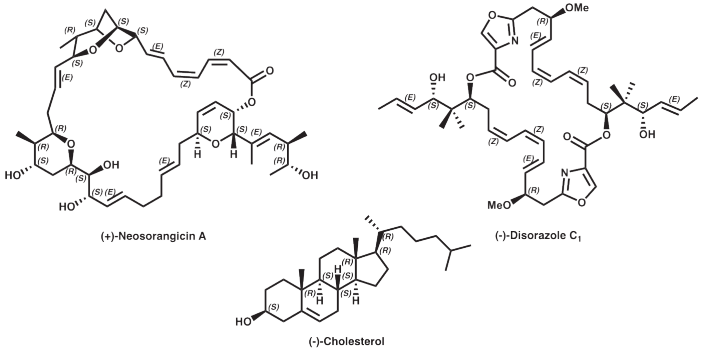
PLENARY LECTURES

TRANSFORMING NATURAL PRODUCT SYNTHESIS INTO THERAPEUTIC PRODUCTS

Dieter Schinzer

Chemisches Institut der Otto-von-Guericke-Universität-Magdeburg, Magdeburg, Germany



The lecture will focus on complex natural product synthesis based on three compounds: (-)-disorazole C_1 a powerful antitumor-active compound, (+)-neosorangicin A a new type of antibiotic, and finally (-)-cholesterol as part of the lipid cocktail for modern mRNA-based Corona vaccines.

ADNP: FROM RESEARCH TOWARD THERAPEUTICS

Illana Gozes

The Elton Laboratory for Molecular Neuroendocrinology, Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Sagol School of Neuroscience and Adams Super Center for Brain Studies, Tel Aviv University, Tel Aviv, Israel.

Background: Regulated by vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP), the essential protein activity-dependent neuroprotective protein (ADNP) and the smallest active fragment, drug candidate NAP were discovered in our laboratory. Recently, the FDA has granted NAP with orphan drug designation and pediatric rare

disease designation for treatment of the rare developmental disorder, the ADNP syndrome. The ADNP syndrome causes intellectual disability (ID), motor dysfunction and autistic traits. Mutated de novo, ADNP is a leading cause for autism/ID.

Objective: The current abstract is designed to update the audience with our most recent results.

Methods and Results: We have recently revealed, that ADNP and related genes are somatically mutated in postmortem aged Alzheimer's disease (AD) brains correlating with increasing Tau pathology, hallmarking AD neurodegeneration (Ivashko-Pachima, Hadar et al., Mol Psychiatry. 2021 May;26(5):1619-1633, recently reviewed in J. Alzheimer's Dis. 2022 Sep 19.). Unexpectedly, we showed extensive tauopathy in post-mortem 7-year-old ADNP syndrome boy (Grigg et al., Transl Psychiatry. 2020 Jul 13;10(1):228). We have further established a genome edited ADNP syndrome mouse, harboring the most prevalent ADNP mutation and showing early tauopathy and protection by NAP (Biol Psychiatry. 2022 Jul 1;92(1):81-95). Mechanistically, ADNP/NAP fortify the nerve cell skeleton/transport system, the microtubules, enhancing Tau-microtubule association and protecting against tauopathy. ADNP mutations disrupt Tau-microtubule interaction, which is protected by NAP. In a new independent study, we further discovered SH3- and actin-binding domains connecting ADNP and SHANK3, through the NAP (NAPVSIP motif) revealing a fundamental shared mechanism underlying autism (Ivashko-Pachima Y et al., Mol Psychiatry. 2022 May 10). In another independent study, we demonstrated NAP enhancement of Tau/sirtuin1-microtubule interaction in human induced pluripotent stem cell-derived neural cells, with sirtuin1 (SIRT1) being a major regulator of healthy aging (Hadar, Kapitansky et al., Mol Psychiatry. 2021 Nov;26(11):6550-6561). Importantly, by direct DNA/chromatin interactions, ADNP regulates hundreds of essential genes hallmarking development/aging with NAP playing

a part, partly through protection of the nuclear envelope, compromised in the face of ADNP mutations (Ganaiem M et al., Cells 2022; 11(19): 2994.).

Conclusions: NAP fortification of ADNP activity, protection against tauopathy and correction of gene expression patterns is predicted to be beneficial in autism and beyond. Davunetide (NAP) is currently developed for the ADNP syndrome by ATED Therapeutics Ltd, Prof. Gozes, Chief Scientific Officer.

Acknowledgement: Supported by ERA-NET Neuron grant ADNPinMed, Drs. Ronith and Armand Stemmer, French Friends of Tel Aviv University and Holly and Jonathan Strelzik (American Friends of Tel Aviv University).

INCLUSION COMPLEXES OF CUCURBITURILS WITH NATURAL ACTIVE COMPOUNDS: TWO CASE STUDIES

Fadwa Odeh

Department of Chemistry, The University of Jordan, Amman, Jordan

The preparation and characterization of CB[7] inclusion complexes with two active compounds originating from natural sources was conducted. CB[7]-Cinn and CB[7]-TQ complexes were studied using optical and spectroscopic techniques in addition to electron microscopic imaging. The aqueous solubility of guest compounds was clearly enhanced upon the addition of CB[7], which provided an initial indication for supramolecular complexation. The complexation stoichiometry and the binding constant of the inclusion complex were determined through a combination of two sets of titration methods, including UV-visible and fluorescence displacement titrations. Moreover, a theoretical evaluation of the CB[7]–Cinn and CB[7]–TQ inclusion complexes stoichiometry and energy measurements was performed. The capability of CBs as drug carriers is discussed.

THERAPEUTIC APPLICATIONS OF ACTIVE PHYTOMEDICINES IN THE PREVENTION OF NEUROLOGICAL DISORDERS

Sidharth Mehan[1], Swesha Chhabra[1], Kajal Sherawat[1], Ghanshyam Das Gupta[2]

[1]Division of Neuroscience, Department of Pharmacology, ISF College of Pharmacy, Moga, Punjab, India

[2]Department of Pharmaceutics, ISF College of Pharmacy, Moga, Punjab, India

Background: The central and peripheral nervous systems, including the brain, spinal cord, and nerves, are all affected by neurological dysfunctions. These are common all across the world. The aetiology is unknown; however, it may or may not result in neuromuscular and motor neuron degeneration, as well as different neuropsychiatric disorders.

Objectives: For the last 13 years, my group at ISF College of Pharmacy has been focusing on: (1) drug discovery screening methods, (2) identification of diagnostic biomarkers, and (3) validation of pharmaceuticals and nutraceuticals for CNS Motor Neuron Diseases (e.g., Multiple Sclerosis and Amyotrophic Lateral Sclerosis), Alzheimer's, Autism, Brain Hemorrhage, Obsessive Compulsive Disorder, Bipolar Disorders.

Methods: These investigations have allowed us to gain competence in using experimental animals to study neurological illnesses. This pursuit has resulted in the development of numerous capabilities in the research of neurogenesis, cell signaling molecules such as ERK, c-JNK, p38MAPK, shh, SIRT-1, JAK-STAT, nrf2/HO-1, GLP-1/IGF-1, PPAR-, PI3K/AKT, mTOR, and Neuronal mitochondria (CoQ10, ETC-Complexes). Our research team works on various projects, including utilizing natural products to treat neurological illnesses. There are presently over 600 neurological illnesses known to exist.

Results: I will explain the pharmacology of active phytoconstituents, given the intense interest in the applications of natural products in the research of

neuroinflammation, neurodegenerative, and neuropsychiatric illnesses. My presentation will demonstrate that: (1) Active phytoconstituents improve neurotoxin-induced experimental models of brain disease by restoring behavioural, molecular, neurochemical, and morphological alterations, and (2) Active phytoconstituents can modulate both cellular and molecular signalling pathways by attenuation of ERK/c-JNK/p38-MAPK, JAK/STAT3, PI3K/Akt-mTOR, and augmentation of SIRT-1, Nrf2/HO-1, SMO-Shh, and mitochondrial ETC-complexes enzymes in neurodegenerative and neuropsychiatric disorders.

Conclusion: As a result, our ongoing initiatives will help to advance preclinical testing of newly identified natural products/moieties and their associated targets for diagnosing and treating neurodegenerative disorders.

MELIORATING EFFECT OF *HELIX ASPERSA* EXTRACT AND ITS MECHANISMS OF ACTION IN EXPERIMENTAL MODEL OF ALZHEIMER'S TYPE OF DEMENTIA

Reni Kalfin[1], Lyubka Tancheva[1], Maria Lazarova[1], Krasimira Tasheva[2], Teodora Taseva[2], Diamara Uzunova[1], Borislav Minchev[1], Polina Petkova-Kirova[1], Valya Grigorova[1], Elina Tzvetanova[1], Almira Georgieva[1], Albena Alexandrova[1], Lyudmila Velkova[3], Pavlina Dolashka[3]

[1]Institute of Neurobiology, Bulgarian Academy of Science, Sofia, Bulgaria
[2]Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]Institute of Organic Chemistry with Center for Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Alzheimer's type dementia (ATD) is a complex neurodegenerative disease with multifactorial etiology, unsatisfactory treatment and necessity of broad-spectrum active substances for cure. Snail mucus from Helix aspersa is a mixture of bioactive molecules with antimicrobial, anti-inflammatory, antioxidant and antiapoptotic effect.

Objective: The effects of snail extract (SE) from Helix aspersa on learning and memory deficits in Alzheimer's type dementia (ATD) induced by scopolamine (Sco) in male Wistar rats were examined and some mechanisms of action underlying these effects were evaluated.

Methods: SE obtained from garden snail Helix aspersa was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Molecular masses of protein in mucus fraction >20 kDa was measured by an Autoflex™III, High-Performance MALDI-TOF. Obtained SE consists of 50% crude mucus extract and 50% fraction containing compounds with MW above 20 kDa. SE (0.5 mL/100 g) was applied orally through a food tube for 16 consecutive days: 5 days before and 11 days simultaneously with Sco (2 mg/kg, intraperitoneally). The changes in spatial learning, short and long term memory processes and exploratory activity of the animals were evaluated behaviorally. In parallel the variation in biochemical parameters: acetylcholine (ACh) and monoamine levels, acetylcholinesterase activity and levels of main oxidative stress markers in the cortex and hippocampus of the experimental animals were determined. Expression of brain-derived neurotrophic factor (BDNF) and cAMP response element-binding protein (CREB) were also evaluated.

Results: SE significantly compensated the memory deficits, observed in dement rats with positive effect on short- and long- term memory processes. This effect of SE was accompanied by inhibition of AChE activity. SE reduced significantly the oxidative stress both in cortex and hippocampus. The acetylcholine, noradrenaline and serotonin levels were enhanced as compared to dement rats. Moreover, multiple SE applications not only restored the depressed by Sco expression of CREB and BDNF, but significantly upregulated it.

Conclusion: The AChE inhibitory activity, moderate antioxidant properties as well as its possibility to enhance acetylcholine and monoamine content in brain structures, related to memory, provide a complex character of the SE treatment. These results were confirmed by principal components analysis,

which demonstrated a very close position between the Sco+snail and control groups, supporting the restorative effect of SE in Alzheimer's type dementia. In conclusion, SE demonstrated complex therapeutic potential in experimental ATD.

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ORAL PRESENTATIONS

MULTI-TARGET STRATEGY USING NATURAL COMPOUNDS FOR EXPERIMENTAL TREATMENT OF ALZHEIMER'S TYPE DEMENTIA

Lyubka Tancheva[1,2], Stela Dragomanova[3], Simona Alexandrova[1], Maria Lazarova[1], Diamara Uzunova[1], Elina Tsvetanova[1], Almira Georgieva[1], Albena Alexandrova[1,3], Miroslava Stefanova[1], Hristian Staykov[4], Yordan Hodzhev[5], Reni Kalfin[1]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Weizmann Institute of Science, Rehovot, Israel*
[3]*National Sport Academy, Sofia, Bulgaria*
[4]*Medical University, Sofia, Bulgaria*
[5]*National Center for Infectious and Parasitic Diseases, Sofia, Bulgaria*

Background: Alzheimer's disease (AD) is one of the most common form of neurodegenerative disorders with high social and medicinal impact. AD multifactorial pathogenesis requires multi-target compounds that can attack more than one biological target. Three natural compounds with rich spectra of biological activities were selected for our investigations: Myrtenal (M) - bicyclic monoterpenoid, Ellagic acid (EA) - polyphenol from pomegranate, and Alpha Lipoic Acid (ALA) - dithiol antioxidant. All three compounds are relatively not much studied in the field of neuroscience.

Objective: To evaluate the effectiveness of M, EA and ALA on experimental model of Alzheimer's type dementia (ATD) in rats.

Methods: ATD was produced by Scopolamine (Sco) treatment for 11 consecutive days in a dose of 2 mg/kg intraperitoneally, applied in adult male Wistar rats. The experimental model of ATD was used after behavioral, biochemical and histological verification. Compounds were administered in effective doses simultaneously with the neurotoxin Sco. Behavioral, biochemical and histological studies were performed at the end of the experiment. Several behavioral tests were used to evaluate changes in the short-term and long-term memory (Step through passive avoidance test, Novel Object Recognition test, T-maze test and Barnes maze test), followed by some histological and biochemical studies.

Results: Neuroprotective effect of Myrtenal on impaired learning, memory and spatial orientation of dement rats is a complex process including antioxidant and neuromodulatory mechanisms of action. The histopathological data point out the localization of Myrtenal neuroprotection towards dementia in the prefrontal cortex.

EA exerted also significant protective effects towards progression of dementia in rats, related to it's strong antioxidant potential and affecting the permeability of blood brain barrier.

ALA can prevent Sco–induced memory impairment and improve spatial orientation via complex neuroprotective mechanisms including antioxidant activity and neuromodulatory capacity. Significant inhibition of AChE activity and correction of aberrant monoamines (dopamine, serotonin and noradrenaline) content in hippocampus and cortex were also documented.

Conclusions: Together with their well-known antioxidant effects, the studied three natural compounds possess additional mechanisms of action contributing to their memory improving effects, namely neuromodulation of the cholinergic and catecholaminergic systems in two main brain structures related to memory.

THE ROLE OF MYOKINES IN THE PHYSIOPATHOLOGY OF HYPERTENSION

Nurettin Aydogdu

Department of Physiology, Faculty of Medicine, İnönü University, Malatya, Türkiye

Skeletal muscle makes up about 40% of the total body weight. Recent studies have identified skeletal muscle as a secretory organ capable of producing cytokines and muscle fiber-derived peptides. Cytokines and peptides released by skeletal muscle are defined as "myokines". Myokines are molecules that play an important role in physiological and pathological functions in

maintaining homeostasis. Myokines can have autocrine, paracrine, and endocrine functions throughout the body. It has been reported that myokines play an important role in muscle adaptation to exercise. In addition, myokines perform physiological functions by interacting with other organs in an endocrine way and are important in mediating the whole body effect. Recent findings show that skeletal muscle-derived myokines protect human health and can treat many diseases.

It has been determined that skeletal muscle plays an important role in the regulation of blood pressure and the development of hypertension. However, little is known about the role of myokines in the regulation of blood pressure and the pathogenesis of hypertension. In this study, we evaluated the role of myokines in hypertension and its complications. Recently, numerous studies have investigated the role of myokines in regulating their effects on hypertension. Here, a review of the most recent findings in the literature and our laboratory results regarding the effects of myokines in the pathophysiology of hypertension are summarized.

Our laboratory studies demonstrated that: i) Physiological dose of chronic irisin treatment did not reduce blood pressure and no significant difference was observed in irisin levels in rats given 150 mg/L dose of Nω-Nitro-L-arginine Methyl Ester Hydrochloride (L-NAME) in drinking water for 3 weeks; ii) It was observed that serum irisin levels were significantly decreased in rats given L-NAME (400 mg/L) with drinking water for 6 weeks; iii) In rats given L-NAME (400 mg/L) with drinking water for 6 weeks, although a decrease in serum myonectin levels was observed, no significant difference was detected in urine, heart, muscle and kidney levels. There was no significant difference in serum, urine, heart, muscle and kidney meteorin-like protein levels.

Recent studies indicate that myokines have important effects in the regulation of blood pressure. When we evaluate our laboratory results and literature data together, the conclusion is made that myokines may play an important

role in the pathophysiology of hypertension. We believe that there is a need for furthur studies on the role of myokines in the pathophysiology of hypertension.

EFFECTS OF MYRTENAL IN EXPERIMENTAL NEURODEGENERATIVE MODELS

Stela Dragomanova[1,2], Maria Lazarova[1], Stoyan Pavlov[2], Desislava Marinova[2], Elina Tzvetanova[1], Albena Alexandrova[1], Almira Georgieva[1], Diamara Uzunova[1], Petya Gavrilova[1], Miroslava Stefanova[1], Maria Papazova[1], Reni Kalfin[1,3], Lyubka Tancheva[1]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria*
[3]*South-West University "Neofit Rilski", Blagoevgrad, Bulgaria*

Background: Plant monoterpenoid Myrtenal (M) is known with its rich biological activities. Numerous beneficial effects of this substance have been already described, e.g., bronchodilatory, anti-inflammatory, antiplatelet and anti-hemolytic, as well as antibacterial (against G (+) pathogens). However, up to now, there is no data about Myrtenal's action on neurodegenerative processes.

Objective: The aim of the study was to evaluate neuroprotective mechanisms of myrtenal in two rat experimental models of neurodegeneration – dementia of Alzheimer's type and Parkinson's disease (PD).

Methods: Dementia was induced in male Wistar rats by scopolamine (Sc) intraperitoneal administration (0.1 mg/kg for 8 days and 20.0 mg/kg on day 9) and PD – via an intrastriatal injection of 6-hydroxydopamine (6-OHDA). Myrtenal was applied i.p. simultaneously with Sc for 9 days or five days prior the 6-OHDA lesion as a pretreatment. Changes in recognition memory and habituation of rats were evaluated via the Novel Object Recognition and Open Field tests in scopolamine-induced dementia protocol. In experimental

PD behavioral investigations included apomorphine-induced rotations count, rotarod test, and the passive avoidance test on the 2nd and 3rd week after the lesion. Brain acetylcholinesterase (AChE) activity, ACh content, noradrenaline (NA) and serotonin (5-HT) levels in induced dementia, as well as dopamine (DA) content in PD model were evaluated. Changes in oxidative status of the brain were measured in both experimental protocols. The histological changes in two brain regions – cortex and hippocampus, were evaluated qualitatively and quantitatively, after M application in Sc-induced damage.

Results: In scopolamine-induced dementia, myrtenal improved recognition memory and habituation, exerted antioxidant effects and significantly increased NA, 5-HT and ACh brain levels, without affecting AChE activity. Its neuroprotective capacity, demonstrated by an increased number of viable neurons in the cortex, was also histologically confirmed. In 6-OHDA induced damage, the monoterpenoid improved learning and memory performance as well as neuromuscular coordination, accompanied by increased dopamine levels and a beneficial effect on oxidative stress.

Conclusions: For the first time, we have demonstrated the neuroprotective potential of myrtenal on two experimental models of neurodegeneration, via at least two different mechanisms – neuromodulatory and antioxidant.

MODULATORS OF OXIDATIVE STRESS AS ANTINEOPLASTIC AGENTS

Luciano Saso

Faculty of Pharmacy and Medicine, Sapienza University of Rome, Rome, Italy

Oxidative stress (OS) plays an important role in neoplastic diseases and inhibitors of nuclear factor erythroid 2-related factor 2 (Nrf2), the master regulator of endogenous antioxidant enzymes, could be useful in their

treatment. However, novel approaches to redox therapies are necessary and the development of reliable biomarkers capable to predict the clinical responses is crucial.

COMBINATORIAL APPROACHES FOR GENERATION OF MULTITARGET-DIRECTED DRUGS AGAINST PARKINSON´S DISEASE

Nikolay Tzvetkov

Department of Biochemical Pharmacology and Drug Design, Institute of Mlecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Neurodegenerative diseases (NDDs) are characterized by chronic, irreversible, and progressive neuronal degradation in the human brain, caused by complex pathophysiological processes, including oxidative stress, excitotoxicity, mitochondrial dysfunction, and others. The current therapy of NDDs, including Alzheimer´s disease (AD) and Parkinson´s disease (PD), is based on the "one molecule-one target" paradigm. These so called "single-target" drugs have an impact on several symptoms in different disease stages, but do not stop the disease progression nor exhibit a neurorestorative effect.
Objective: To combat the multifactorial nature of PD, the focus is now shifted toward development of small molecules that modulate more than one therapeutic CNS target – the so called multi-target-directed ligands (MTDLs). In this study, we present the *in vitro* efficacy of newly discovered MTDLs.
Methods: The binding modes at PD-related biological targets of newly reported single MAO B or dual acting MAO A/B inhibitors were computed and analysed using the HYdrogen DEssolvation (HYDE) scoring function in SeeSAR molecular modelling platform.
Results: To confirm the predicted binding activities (proof-of-concept), the small molecule-based drugs were found to be effective as α-synuclein

inhibitors, in addition to their initially discovered biological properties.

Conclusion: A structure-based multi-step molecular modelling was used to estimate a set of unique first-in-class generation MTDLs, acting as single monoamine oxidase B (MAO-B) or dual MAO-A/B, acetylcholinesterase (AChE), and α-synuclein inhibitors. The investigated first-in-class generation of MTDLs offer the possibility for further in vivo evaluation of these compounds as potential anti-parkinsonian agents.

Acknowledgement: The National Science Fund of Bulgaria (grant KP-06-OPR 03/8) is gratefully acknowledged.

ANTIMICROBIAL PEPTIDES FROM THE MUCUS OF THE GARDEN SNAIL *CORNU ASPERSUM* – MECHANISM OF ACTION

Pavlina Dolashka[1], Lyudmila Velkova[1], Aleksandar Dolashki[1], Karina Marinova[1], Peter Petrov[1], Ventsseslav Atanasov[1], Dimiter Kaynarov[1], Nevena Ilieva[2], Elena Lilkova[2], Momchil Kermedchiev[3], Mihaela Belouhova[4], Ekaterina Krumova[5], Yana Topalova[4], Maria Angelova[5], Leandar Litov[6], Hristo Najdenski[5]

[1]*Institute of Organic Chemistry with Center for Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Information and Communication Technologies at the Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*University hospital Alexandrovska, Sofia, Bulgaria*
[4]*Faculty of Biology, University of Sofia "St. Kliment Ohridski", Sofia, Bulgaria*
[5]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[6]*Faculty of Physics, University of Sofia "St. Kliment Ohridski", Sofia, Bulgaria*

The snail slime (mucus) has many functions in the animal, such as adhesive, emollient, moisturizing, lubricant, and defense. Recently, snail mucus has been applied in human medical and cosmetics. The antibacterial activity of the mucus of garden snail *Cornu aspersum* has been proven both *in vitro* and

also *in vivo* in a number of clinical studies.

The primary structure of more than 50 newly identified antimicrobial peptides (AMPs) in the mucus on the basis of their MALDI MS/MS spectra has been established and their antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* has been investigated.

Understanding of the mechanism of action AMPs on bacterial cells requires detailed knowledge of how AMPs interact with bacterial membranes. Based on large-scale simulations we hypothesize the multistage nature of the antibacterial activity of peptides and the formation of mixed peptide clusters as a transport and concentration agent to deliver the active ingredients to the target bacterial membrane. Stepping on the simulation results, the antimicrobial activity of a number of two- and three-component peptide mixtures was probed and confirmed experimentally. The antibacterial test confirmed the inhibitory effect and the synergistic effect of different combinations of peptides against *E. coli 3584* and *B. subtilis*.

Changes in bacterial structure and metabolic activity, investigated by SEM, fluorescence and digital image analysis, present strong inhibitory effects of these AMPs in superficial and deep inoculations of *E. coli*.

The clinical efficacy of these compounds has been investigated through *in vivo* trials in both animal models and humans. Wound studies show the presence of bacterial and fungal infections, which are a major complication of diabetic wounds and cause serious harm to patients. Traditional therapies and natural products have been used to stimulate the wound regeneration process with promising results. Antibacterial and antifungal activity of *C aspersa* snail mucus in patient wound isolates, as well as the regenerative effect, have been demonstrated.

Acknowledgments: This work is supported in part by the BSF grant KP-06-OPR 03-10/2018 and the Grant D 01-217/30.11.2018 and agreements D 01-323/18.12.2019, D 01-358/17.12.2020, D 01-278/03.12.2021 under NRP

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DEVELOPMENT AND MOLECULAR CHARACTERIZATION OF BIOMARKERS IN THE DIAGNOSIS AND PREVENTION OF MOTOR NEURON AND NEUROPSYCHIATRIC DISEASES

Sidharth Mehan[1], Sarthak Sharma[1], Aditi Giri[1], Ghanshyam Das Gupta[2]

[1]Division of Neuroscience, Department of Pharmacology, ISF College of Pharmacy, Moga, Punjab, India
[2]Department of Pharmaceutics, ISF College of Pharmacy, Moga, Punjab, India

Background: Neurodegenerative diseases (NDD) such as Multiple Sclerosis (MS) and Amyotrophic lateral sclerosis (ALS) are motor neuron demyelination disorder that causes spinal nerve degeneration. It is a subset of disease which primarily affects the brain stem, motor cortex, spinal cord, and corticospinal tract. Besides, neuropsychiatric disorders (NPD) such as Autism, OCD and bipolar disorders prevalent throughput the globe. Worldwide, several people are suffering from NDD and NPD-associated several neurocomplications.

Objectives: The increase in the number of these patients with NDD and NPD has led to increasing demand for effective therapy and a particular diagnosis which can prevent this motor neuron dysfunction. There are limited studies regarding molecular biomarkers to diagnose and treat these neurological abnormalities. Thus, there is no evidence regarding pre-clinical or clinical research data associated with these molecular markers and test drugs,

especially in specific brain diseases like MS, ALS, Autism and OCD.

Methods: There are currently several diagnostic tests available to diagnose these disorders, including blood and urine tests are laboratory tests that analyze blood and urine samples that include spinal tap lumbar puncture, electromyogram, nerve conduction study, MRI, etc. The major limitations of all these diagnostic tests are quite laborious, intrusive, and costly. As a result, people avoid or delay getting diagnosed, causing theirsymptoms to worsen. Furthermore, no single test can provide a definitive diagnosis of MS, ALS, Autism and OCD, leading to the disorder's progression and worsening. This could be prominent in the mortality rate. However, the most simple and most accessible option might be to identify cellular and molecular markers as well as quantify heavy metals thatquickly accumulate in the saliva, tears, nails and hairs of MS, and ALS patients, which sounds promising for early detection and prevention.

Results: Our preliminary laboratory findings support using cellular and molecular target proteins as diagnostic biomarkers in blood plasma, CSF and urine samples and their neuroprotective role via signalling modulators. Therefore, preclinically, we investigate a further study to establish and validate these biomarkers in MS, ALS, Autism and OCD experiments. Considering the complexity of the disease diagnosis, there is a dire need to have a reliable marker for MS, ALS, Autism and OCD patients.

Conclusion: A laboratory test may be developed based on different markers clubbed together. Performing multiple tests simultaneously to evaluate the biomarkers in various biological samples including blood serum, CSF, saliva, urine, toenail, and hair has an advantage over a single biomarker test. This study will let the researcher and clinicians quantify the levels of pathogenic proteins in people living with MS, ALS, Autism and OCD Hence, combining these biomarkers will emerge an efficient method of detecting MS, ALS, Autism and OCD in the early stages.

RESPONSE-RELATED POTENTIALS AND OSCILLATIONS: APPLICATION TO AGING RESEARCH

Vasil Kolev, Juliana Yordanova

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Response-related potentials (RRPs) are a specific class of event-related brain potentials. The characteristics of RRPs is that the particular event that triggers them is the movement rather than a sensory stimulus or any other environmental or internal event. Hence, RRPs are computed from the electroencephalogram (EEG) in order to illuminate the neurophysiological mechanisms associated with movement generation (preparation, initiation and production). They are maximally expressed at pre-motor, motor and sensorimotor cortical regions contra-lateral to the side of movement or response and are typically analyzed at these areas. However, movement generation is usually accompanied by processes of cognitive control including attention, movement/motor response selection, performance monitoring, error detection, and performance adjustment and optimization. Thus, RRPs at pre-frontal, medial frontal, central and parietal regions can be explored to reflect such processes.

RRP elicitation and analysis follows the approaches required for event-related potentials. Thus, in experimental conditions, it is necessary to employ a multitude of repeated movements/responses in order to acquire a sufficient number of single trials to reduce signal-to-noise ratio after averaging. Likewise, RRPs can be analyzed in the time, frequency and time-frequency domains. While in the past, time-domain analysis was basically used, advanced approaches for time-frequency decomposition are increasingly applied. The major advantage of time-frequency decomposition methods is that they can extract and reveal important oscillatory components of RRPs. The application of such approaches (e.g., digital filtering, wavelet decomposition, ERD/

ERS, etc.) has highlighted novel neural aspects of movement generation as demonstrating the physiological significance of frequency-specific activities from delta, theta, alpha, beta and gamma ranges that play important roles for movement generation and regulation. Also, time-frequency approaches allow analysis of cortical connectivity during movement production and motor-related networks. It is to be noted that RRPs are best extracted after spatial enhancement of the EEG.

It will be demonstrated how applying time-frequency decomposition and oscillations can reveal relevant modulations in functional connectivity and mechanisms of motor generation performance monitoring induced by aging in humans.

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CHANGES IN VISUO-SPATIAL FUNCTIONS IN PATIENTS WITH HYPOTHYROIDISM AFTER TREATMENT WITH LEVOTHYROXINE

Kalina Racheva[1], Nadejda Bocheva[1], Bilyana Genova[1], Miroslava Stefanova[1], Simeon Stefanov[1], Emil Natchev[2]

[1]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Department of Endocrinology, Medical University, Sofia, Bulgaria

Background: In a previous study (Bocheva et al., 2022), we investigated the effect of thyroid deficiency on the visuospatial abilities of newly diagnosed patients with hypothyroidism. We explored its impact on the performance of visual tasks related predominantly to the processes in the dorsal and ventral pathways. The study's results showed subtle deficits in temporal and spatial integration of visual information, implying deficient processing in the ventral system and reallocation of spatial attention under cognitive load.

Objective: Using the same stimuli, procedure, and participants as in the previous study, we aimed to investigate whether treatment with levothyroxine improves the visuospatial abilities of the patients.

Methods: Two different tasks were employed. The first one required the location of the center of radial patterns and supposedly relied predominantly on the dorsal pathway. In the second task, the participants had to discriminate the shape of dot patterns – radial or circular. Its performance relies most on the processes in the ventral system. In both tasks, we used Glass patterns as stimuli – patterns with paired dots (dipoles) separated by a fixed distance whose spatial relations convey different global forms. We varied the coherence of the stimuli (their deviation from a pre-defined form) and the lifetime of the dipoles.

Seventeen out of the 26 patients participating in the previous experiments took part in the study. The control group contained 21 out of the 26 former participants.

Results: The comparison of the results from the two studies shows improvements in the patient performance in discriminating shapes with no difference with the controls in dynamic conditions and superior performance of the patients in static conditions. The difference between the two groups in discriminating circular from radial patterns also disappears.

Similarly, the accuracy in localizing the center of radial patterns is similar to the control group. Contrary to the previous study, no difference is observed in the patient group depending on the center position. The higher accuracy of the patients than the controls for the radial patterns is preserved.

Conclusions: The treatment with levothyroxine improves the visuospatial abilities of patients with hypothyroidism. This effect might be related to the role of levothyroxine treatment in the stimulatory effects of dopamine and serotonin and its restoration.

MECHANISM OF PROTECTIVE ACTION OF GARDEN SNAIL MUCUS IN A MODEL OF ALZHEIMER'S DISEASE – PROTEOMIC ANALYSES

Lyudmila Velkova[1], Ventseslav Atanasov[1], Lyubka Tancheva[2], Alexander Dolashki[1], Reni Kalfin[2], Diamara Uzunova[2], Pavlina Dolashka[1]

[1]*Institute of Organic Chemistry with Center for Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Alzheimer's disease is one of the most complicated neurodegenerative diseases, and several hypotheses have been associated with its development and progression. Nowadays, research is focused on the discovery of new therapeutic agents with more than one target for pharmacological action.

The mucus of the garden snail is a complex biological mixture containing a various bioactive compounds with different molecular weights and properties. Our investigations of the mucus extract from *Helix aspersa* showed a presence of antimicrobial peptides with molecular weight (Mw) below 10 kDa as well proteins with a Mw above 20 kDa, with antimicrobial, antioxidant activity and regenerative properties, which were detected by mass spectrometry. Additional, the metabolites with low molecular weight such as allantoin, glutathione, glycolic acid, choline and amino acids were found. Some of them also possess antioxidant and antibacterial activity. On the other hand, the choline plays an important role as a precursor for the synthesis of the neurotransmitter ACh, as well as in participates in lipid transport and metabolism.

We used scopolamine model for assessment of potential neuroprotective effect of mucus extract from *H. aspersa* on neurodegenerative processes *in vivo*.The experimental rats were divided on three groups: a control group of healthy rats, a scopolamine group (treated with scopolamine) and an experimental group treated with scopolamine and snail extract together. Two

major memory-related brain structures (hippocampus and prefrontal cortex) were isolated. In order to identify changes in the expression of proteins in cortex and hippocampa, before and after treatment with scopolamin and mucus extract a proteomic analysis was performed, including two-dimensional gel electrophoresis (2D-PAGE), mass spectrometry and bioinformatics. Using MASCOT Peptide Mass Fingerprint, and Melanie Coverage 9.2 software, proteins in the cortex and hippocampus that change their expression have been identified. Our results show that treatment with the mucilage extract leads to an increase in the expression of various proteins such as Heat shock protein 90 kDa, Peroxiredoxin-6, Tubulin α and β, Synapsin, Malate dehydrogenase, and Ubiquitin carboxyl-terminal hydrolase isozyme L1, but other proteins reduce their expresion – β –Actin, Phosphoglycerate mutase 1, and etc.

The positive effect of the *H. aspersa* mucus is a result of the synergistic action of compounds with antioxidant capacity and antimicrobial and regenerative properties.

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ARTERIAL STIFFNESS PARAMETERS IN PATIENTS WITH ARTERIAL HYPERTENSION WITH AND WITHOUT STEATOHEPATITIS

Vesela Georgieva[1,2], Anelia Dimitrova[1,3]

[1]Department of Physiology, Pathophysiology and Pharmacology, Medical Faculty of the Trakia University, Stara Zagora, Bulgaria
[2]Medical Center Stoyan Kirkovich Stara Zagora, Bulgaria
[3]Department of Pathophysiology, Medical University Pleven, Pleven, Bulgaria

Background: Non-alcoholic steatohepatitis (NASH) is common comorbidity in patients with essential arterial hypertension (AH), because both of them are related with metabolic syndrome and visceral (metabolic active) adiposity. On the other hand, dyslipidemia and the impairment of liver function observed in steatohepatitis are risk factors for the development of cardiovascular diseases.

Objective: Therefore, the aim of our study is to compare arterial stiffness parameters in obese hypertensive patients with and without NASH.

Methods: Based on the results of biochemical and ultrasound screening (lipid profile, Alanine Aminotransferase, Aspartat Aminotransferase and ultrasound of abdominal organs), 170 patients with known arterial hypertension and obesity are divided into three groups - patients with normal liver, with non-alcoholic hepatic steatosis and non-alcoholic steatohepatitis. Aortic profile, some arterial stiffness parameters (augmentations pleasure, augmentations index, augmentations index 75) and subendocardial viability ratio (SEVER) are measured with cuff-based arterial tonometry in all patients. After height and weight measurements, BMI is calculated using the usual formula.

Results: 62 of the examined hypertensive patients had no liver damage, 64 had non-alcoholic hepatic steatosis, and 44 had non-alcoholic steatohepatitis. Regardless of the satisfactory medical control of arterial pressure in all groups, the group of patients with AH and steatohepatitis is distinguished

by a statistically significant higher augmentation pressure and augmentation index. There are no significant differences of subendocardial viability ratio in the three groups, but it is lower in patients with higher body mass index. In addition, the patients of our sample with a SEVER below 110%, the symptoms of heart failure were observed statistically significantly more often.

Conclusions: Patients with coexistence of AH and NASH have increased arterial stiffness. This can be considered as a consequence of the atherogenic potential of dyslipidemia, metabolically active obesity and insulin resistance. The oxygen supply-demand myocardial ratio does not depend on the liver impairment, but it is related with BMI. In patients with AH and obesity low SEVER could lead not only to myocardial ischemia, but also to clinically manifestation of heart failure. This finding can explain why also metabolically not active obesity leads to cardiomyopathy. Based on our finding we can recommend follow-up of arterial stiffness in hypertensive patients with obesity and steatohepatitis and follow-up of SEVER in hypertensive patients with BMI over 35 for the purposes of diagnosis and prevention.

AORTIC WALL AND PERIAORTIC ADIPOSE TISSUE CHANGES IN OBESE RATS SUPPLEMENTED WITH MELATONIN

Dariya Chivchibashi-Pavlova, Diyana Kyuchukova, Kameliya Bratoeva

Department of Pathophysiology, Faculty of Medicine, *Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria*

Background: To date, cardiovascular disease (CVD) remains the leading cause of death globally. Aortic wall and periaortic adipose tissue (PAAT) dysfunction predicts short- and long- term CVD morbidity and mortality. Obesity contributes to PAAT dysfunction and increases the risk of a broad spectrum of pathologies with vascular manifestation. The latter are most commonly

pathogenically associated with chronic oxidative stress, pro-inflammatory state, and hypoxia. Hence, the pineal gland hormone melatonin as an antioxidant and anti-inflammatory agent is expected to act vasoprotective.

Objectives: In the present study we evaluated the effect of melatonin supplementation on PAAT and aortic wall thickness via assessing the cross-sectional associations of abdominal obesity with aortic intima-media thickness in diet-induced obesity rat model.

Methods: We used a model of high-fructose diet (HFD-12 weeks, 20 % glucose-fructose corn syrup) in male Wistar rats divided into four groups (n=8): control; HFD; HFD and melatonin supplementation ("per os"-4 mg/kg/24h); control and melatonin supplementation group. All rats received standard rodent diet and tap water. Zoometric measurements and Lee index were investigated. Routine histologic morphometric analyses of the abdominal aorta were performed using Aperio Image Scope software. Histopathological changes in PAAT were also observed.

Results: The results showed significantly elevated body weight (Lee index) and increased wall thickness of the *a.abdominalis* with pathomorphological changes in the vessel wall and PAAT in HFD rats compared to the control group.

Conclusions: Obesity is tightly linked to the development of aortic and PAAT dysfunction. In particular, HFD administered to Wistar rats leads to pathomorphological and morphometric alterations in the abdominal aorta, which are the main diagnostic criteria of vascular and PAAT dysfunction. Melatonin supplementation reverses these changes. In particular, melatonin supplementation group demonstrated increased amounts of brown adipose tissue foci in PAAT, whereas HFG presented with PAAT exclusively represented by hypertrophic white adipose tissue with inflammatory changes.

STUDY OF AGE-DEPENDENT EFFECT OF MELATONIN DEFICIENCY ON BEHAVIOR: THE ROLE OF CHAPERONE PROTEINS AND OXIDATIVE STRESS

Desislava Krushovlieva[1], Simeon Georgiev[1], Petja Ivanova[1], Zlatina Petkova[1], Tzveta Stoyanova[1], Irina Georgieva[2], Rumyana Tzoneva[2], Jana Tchekalarova[1]

[1]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Ageing is a natural phenomenon that involves many biological changes, including increased oxidative stress, DNA damage, protein misfolding, and mitochondrial dysfunction. On other hand, melatonin is a powerful antioxidant that involved in the control of important physiological processes including chaperone protein (Hsp) expression.

Objective: The present study was designed to ascertain the role of melatonin system on aging process. For the purpose, we evaluated age-dependent effect of melatonin deficiency in rat on anxiety and cognitive behavior as well as their correlation with Hsps 70 and 90 in the frontal cortex and the level of oxidative stress in the hippocampus.

Methods: The experiments were performed on male Wistar rats at three ages: young adults (3-6 months), middle-aged (14-17 months) and old (18-21 months). The motor activity and anxiety were assessed by the elevated plus maze (EPM) test and the light dark (LD) test. Cognitive function was evaluated by the object recognition *test* (*ORT*). Brain homogenates from the frontal cortex (FC) and the hippocampus was used for biochemical analysis by ELISA method.

Results: The motor activity was significantly decreased with aging. Young adult 3-month-old rats demonstrated the highest motor activity (total distance in the EPM) compared to the 14- and 18-month-old rat ($p < 0.05$). Age-related elevation of the level of anxiety was also detected (distance in open

arms, time in open arms and number of entries in the EPM; latency and time in light in the LDT) ($p < 0.05$).

Pinealectomy decreased the anxiety level in the youngest and the oldest group of rats compared to age-matched sham rats ($p < 0.05$).

Age-dependent changes in the expression of Hsp 70 and Hsp 90 was detected in the FC. The old sham group had diminished expression of Hsp 70 and 90 compared to the youngest and middle aged rats ($p < 0.05$). Pinealectomy exerted age-dependent changes on Hsp 70 and 90 and oxidative stress, respectively. The middle-aged rats with pinealectomy had decreased expression of Hsp in the FC, diminished activity of SOD and elevated level of MDA in the hippocampus ($p < 0.05$).

Conclusions: Our results showed that behavioral changes (anxiety and cognition) in aging are associated with decreased expression of the Hsp 70 and Hsp 90 in the FC and increased oxidative stress in the hippocampus. Melatonin deficit in young adult rats impairs behavioral responses associated with anxiety and memory while this condition is vulnerable for Hsp expression in the FC and oxidative stress in the hippocampus of the middle-aged rats.

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CENTRAL HAEMODYNAMIC PARAMETERS: EFFECT OF AGE AND GENDER

Rene Mileva-Popova, Nikolay Stoynev, Peter Ivanov, Nina Belova

Department of Physiology and Pathophysiology, Medical University, Sofia, Bulgaria

Background: Cardiovascular diseases are the leading cause for morbidity, impaired quality of life and mortality all over the world. Age is a major, yet, non-modifiable cardiovascular risk factor.

Objective: To further clarify the impact of age and gender on central hemodynamic parameters obtained from non-invasive tonometric pressure waveforms.

Methods: Seventy clinically healthy subjects were divided into 4 groups according to their age and gender: F20 (females, 21 y/o, n=21), M20 (males, 22 y/o, n=22), F50 (females, 59 y/o, n=15) and M50 (males, 47 y/o, n=12). Pulse wave analysis by applanation tonometry (Sphygmocor, AtCor, Australia) was applied for non-invasive assessment of central aortic pressures. Several important derivative indices were computed: the Augmentation index (AIx75) calculated as the ratio Augmentation pressure/Pulse pressure in a percentage, corrected for heart rate 75 beats/minute, the Amplification ratio (AR) calculated as the ratio Radial pulse pressure/Central pulse pressure, the Rate pressure product (RPP) or the product Heart rate × Brachial systolic pressure and the Subendocardial viability ratio (SEVR) that is the ratio Diastolic pressure time interval/Systolic time interval in a percentage. ANOVA and regression statistical analysis were utilized for data comparison between the experimental groups. A cutoff value of 0.05 was accepted for P.

Results: Both the aortic systolic and pulse pressures were significantly higher in the 50 y/o groups as compared to the young individuals (in mm Hg: F20 95 ± 2 and 26 ± 1; M20 100 ± 2 and 33 ± 1; F50 119 ± 4 and 44 ± 3; M50 120 ± 3 and 42 ± 3 respectively; $p<0.01$). This finding was supported by the AIx75 values, a well-known marker of arterial stiffness (in %: F20 -2 ± 3; M20 -8 ± 3; F50 30 ± 3; M50 14 ± 4 respectively). The AIx75 was highest in F50 ($p<0.01$) vs. all groups and higher in M50 vs. the young individuals showing the largest alterations to appear in the middle-aged females as a consequence to both ageing and the loss of the protective estrogen effect. The AR data were correspondingly indicative for the development of functional and morphological alterations in the large arterial tree. AR was lowest in F50 group and markedly lower in M50 as compared to the young individuals

($p<0.01$). In support to the above findings was the highly significant correlation observed between the BRPP as a measure of ventricular load and SEVR, the index of myocardial vulnerability ($p<0.0001$).

Conclusions: Our data support the views for the negative impact of ageing and gender on central hemodynamics and point to the necessity of the implementation of a battery of non-invasive indices for better primary prevention in cardiovascular practice.

POSTER PRESENTATIONS

ANTIOXIDANT POTENTIAL OF SNAIL EXTRACT (*CORNU ASPERSUM*) IN TWO EXPERIMENTAL MODELS OF NEURODEGENERATIVE DISORDERS

Elina Tsvetanova[1], Albena Alexandrova[1,2], Almira Georgieva[1], Maria Lazarova[1], Reni Kalfin[1], Diamara Uzunova[1], Borislav Minchev[1], Pavlina Dolashka[3], Lyudmila Velkova[3], Alexander Dolashki[3], Lyubka Tancheva[1]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*National Sports Academy, Sofia, Bulgaria*
[3]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Background: Oxidative stress induction is accepted as one of the main underlying causes of pathology of various diseases, including neurodegenerative ones. Treatment with natural antioxidants is one of the promising strategies for prevention and mitigation of neurodegenerative impairment. Fresh snail extract is a rich source of biologically active substances with antioxidant, anti-inflammatory, antibacterial, antiviral, immune-stimulating and regenerative properties.

Objective: The aim of the study was to test and compare the therapeutic potential of standardized snail extract **(**SE**)** on two experimental models of neurodegenerative disorders - Alzheimer's type dementia (AD) and Parkinson's disease (PD).

Materials and methods: Fresh mucus was collected from snail *Cornu aspersum*, purified and standardized. Scopolamine was applied (Scop. 1 mg/kg, i.p., 11 days) to adult male Wistar rats. Animals were treated for 11 days with SE orally (0.5 ml/100 g), simultaneously with scopolamine. On the 1 and 5 and 12 day animals were subjected to a behavioral test for evaluation the dynamic of changes in their learning and memory (Step through test). On the 12 day main brain structures, related to memory cortex and hippocampus were extracted. The experimental rat PD model was induced via i.c.v. 6-OHDA administration. Six days before and seven days after striatal lesion,

SE was applied (altogether 13 days) orally. On the 1st, 2nd and 3rd week post lesion animals were subjected to behavioral tests for motor coordination (Rota rod test). After the treatments and behavioral tests performance, the brains were processed for measurements of changes in oxidative status (lipid peroxidation and glutathione levels, and antioxidant enzyme activities superoxide dismutase, catalase and glutathione peroxidase). Also changes in brain acetylcholinesterase (AChE) activity were measured.

Results: SE applied at both animal models (AD and PD) significantly reduced brain LPO and restored the superoxide dismutase activity increased by neurotoxins- 6-OHDA and Scopolamine respectively. The established effect of SE on oxidative status was accompanied by significant inhibition of brain AChE activity (by 38% vs control). Additionally, in AD rats, SE also led to a significant recovery of brain GSH levels, which were reduced by Scop treatment.

Conclusion: Established beneficial effect of SE on the experimental development of PD and AD suggest its successful application against neurodegenerative diseases and deserves more studies as promising strategy.

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ALPHA LIPOIC ACID IN ALCOHOL-RELATED BRAIN DAMAGE - A POTENTIAL CURE?

Hristian Staykov[1], Kalina Koleva[1], Lyubka Tancheva[2], Rumen Nikolov[1]

[1]*Department of Pharmacology and Toxicology, Faculty of Medicine, Medical University, Sofia, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Background: Mental and behavioral disorders due to alcohol use can directly and indirectly lead to alcohol-related brain damage (ARBD) and over 20 other diseases. Thus, they pose a problem of epidemic proportions and exert an enormous disease burden (in terms of health, social, financial, and legal factors) on the people who develop them, their loved ones and society as a whole. Therefore, high-quality research is needed in order to develop effective preventive and therapeutic strategies. Pharmacotherapy with alpha-lipoic acid (ALA) could prove to be an effective strategy.

Objective: To make the hypothesis that ALA can exert preventive and/or therapeutic effects in ARBD.

Methods: Electronic search strategies were developed and undertaken and relevant articles were selected and reviewed.

Results: ALA has antioxidant, neuroprotective and anti-inflammatory effects. This makes it a prospective pharmacological agent in the treatment and prevention of ARBD.

Conclusions: The results confirm that ALA could interfere with and potentially remediate ARBD pathogenesis pathways. Future research can further elaborate on this and its potential translation into clinical practice.

DIFFERENCES IN SPECTRAL CHARACTERISTICS OF HEART RATE AND BLOOD PRESSURE EVOKED BY ETA OR ETB RECEPTOR INHIBITION IN SHR

Petya Markova, Radoslav Girchev

Medical Faculty, Medical University, Sofia, Bulgaria

Background: The endothelins plays essential role in the regulation of cardiovascular function. Its effects occur through the activation of two main receptor subtypes – ETA and ETB. The aim of the current study was to investigate participation of different type endothelin receptors on fast mechanisms of cardiovascular regulation by means of spectral analysis of interpulse interval (IPI) and arterial blood pressure (ABP) in spontaneously hypertensive rats (SHR).

Methods: The experiments were carried out on 12-14-week-old, conscious, males, normotensive Wistar rats (WR, n=8) and SHR (n=8) in control period and during selectively blockade of ETA or ETB by intravenous infusion of BQ123 in dose 16.4 nmol.kg^{-1}.min for ETA receptors, by BQ788 at a dose of 1 mg.kg^{-1}.h for ETB receptors. The SHR group included animals with systolic arterial blood pressure above 160 mmHg. In the arterial pressure (AP) wave, through the Acqknowledge 5 software, the mean values of systolic (SAP), diastolic (DAP), mean (MAP) arterial pressure and interpulse interval (IPI) was determined. By spectral analysis (FFT algorithm), in the LabView graphical programming environment, spectrograms for investigated cardiovascular variables were received and spectral power (P) were studied in typical for rat's spectral bands: LF (20-195); MF (195-605); HF (605-3000) mHz.

Results: We have established reduced power of the humoral mediated, LF and of the sympathetic conditioned MF fluctuations of the cardiovascular variables in SHR in comparison to WR, $p<0.05$. Selective blockade of ETA

receptors did not change mean values of SAP, DAP, MAP and IPI in WR in difference to SHR in which SAP decreased slightly but significant from 183.4±2.5 to 176.1±2.1 mmHg, $p<0.05$. BQ-123 application in both WR and SHR led to increase of PLF and PMF in the IPI spectrograms, ($p<0.05$). Selective blockade of ETA not affects spectral characteristics in ABP in WR but in SHR increased PMF in SAP spectrograms from 1.24±0.9 to 1.92±0.26 $mmHg^2$, DAP from 1.58±0.20 to 2.15±0.36 $mmHg^2$, and MAP from 1.88±0.15 to 2.57±0.28 $mmHg^2$, $p<0.05$. The selective blockade of the ETB receptors did not change mean values of ABP and IPI in WR as well in SHR. In WR administration of BQ788 did not influence power of fast oscillation in either ABP or IPI. However, in SHR it caused decrease in the power of MF and HF oscillations in ABP spectrograms. The PMF in SAP, DAP and MAP spectrograms decreased with 58.3; 71.2 and 82.6%, respectively, $p<0.01$. The PHF in the SAP, DAP and MAP spectrograms were decreased by 63.4; 71.2 and 82.6%, $p<0.01$.

Conclusions: The endothelins by ETA receptors have a modulating effect on fast oscillation of IPI in Wistar rats as well as in SHR. Only in SHR endothelins by both receptor subtypes influence sympathetically mediated fluctuation of ABP but, however, exhibit opposite effects. Through ETB receptors in SHR endothelins modulate and fast respiratory oscillation of ABP. In SHR endothelins participate in the regulation of fast variation of arterial blood pressure.

INFLUENCE OF ARIPIPRAZOLE ON EXCITATION-CONTRACTION COUPLING IN GASTRIC SMOOTH MUSCLES OF RAT

Natalia Prissadova[1], Iliana. Stefanova[1] Valentin Turiyski[1], Rayna Ardasheva[1], Viktor Yotov[1], Damyanka Getova, Atanas. Krustev[2]

[1]*Department of Medical Physics and Biophysics,Medical University, Plovdiv, Bulgaria*
[2]*Medical College, Trakia University, Stara Zagora, Bulgaria*

Background: Aripiprazole is an atypical antipsychotic drug that exhibits simultaneously agonistic, partial agonistic and antagonistic properties regarding various types of dopamine and serotonin receptors. Interacting with different receptor structures, it can influence the function of tissues and organs outside the CNS, including the gastro-intestinal motility.

Objective: The study aimed to investigate the effects of aripiprazole on the cellular mechanisms influencing the excitation-contraction coupling in the gastric smooth muscles (SM) of a rat.

Methods: We have tested the mechanical and bioelectrical properties of isolated SM tissue from rat stomach. The contractile activity and reactivity of the samples were isometrically recorded in tissue bath filled with Krebs solution at 37°C; the bioelectric activity was recorded using the single sucrose gap technique.

Results: Aripiprazole ($1x10^{-5}$mol/L) produced a two-phase contraction/relaxation effect. The contractile phase (5-7 minute of duration) was inhibited with 10^{-6} mol/L atropine. Premedication of SM tissues with acetylcholine ($1x10^{-6}$ mol/L) significantly reduced the duration and strength of the aripiprazole-induced contraction. The relaxation phase was uninfluenced by atropine and reached a maximum value in about 20 minutes. The magnitude of the aripiprazole-induced relaxation was reliably reduced in the presence of acetylcholine and a following treatment of the preparations with $5x10^{-5}$mol/L

SQ 22536 (an inhibitor of adenylate cyclase (AC) activity) and $5x10^{-5}$mol/L KT 5720 (a selective inhibitor of protein kinase A - PKA).

Conclusions: We hypothesize that aripiprazole influences M_3 cholinergic receptors and induces the initial contractile phase. The beta-gamma complex of the receptor-activated G_s protein activates some subtypes of AC in the presence of $G_{\alpha s}$. This increases the cytosolic cyclic adenosine monophosphate level and activates PKA. By phosphorylating various proteins, the latter reduces phasic contraction amplitude, bringing about lasting SM relaxation.

RPM-SIMULATED MICROGRAVITY AFFECTS CARRAGEENAN-INDUCED INFLAMMATION AND BIOCHEMICAL AND HORMONAL INDICES IN RATS

Viktor Yotov[1], A. Mihaylova[2], I. Kostadinov[3], Rayna Ardasheva[1], Valentin Turiyski[1]

[1]Department of Medical Physics and Biophysics, Medical University of Plovdiv, Plovdiv, Bulgaria.
[2]Department of Pharmacology,Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv,Bulgaria
[3]Department of Pharmacology and Clinical Pharmacology, Medical Faculty, Medical University of Plovdiv, Plovdiv, Bulgaria

Background: Limited number of projects of investigation on Random positioning machine (RPM) simulated microgravity effects in experiments with animals have been reported. A few main methods are in use in this term and most widely presented is hindlimb unloading, but it impose restrictions in many aspects.In this reason development of alternative models can open more opportunities in assessment of microgravity effects on living organisms. Such perspective model is RPM adapted for physiological experiments with rats.

Objective: The present study aimed to evaluate whether RPM-simulated microgravity affects carrageenan-induced local inflammation in rats and how it correlates with changes in some biochemical indices and hormones

established during the experiment.

Methods: Two groups of male Wistar rats – "S-control"(n=4) and RPM-simulation group (n=4) were used in experiments: the animals from first ("S"–stress control) group stayed inside static experimental capsules; the RPM-simulation group was exposed to randomized rotation in specially designed RPM. Experiment lasts 120 hours for both groups. After the end of the experiment blood was taken from animals and serum was separated. Following indices were measured: biochemical – hepatic transaminases (AsAT, AlAT), serum amilase, cholinesterase, creatinine, glucose and urea. Hormonal assessment was based on serum levels of thyroid-stimulating hormone (TSH), cortisol and aldosterone. One hour after blood collection all the animals were treated with single subplantar injection of 1 % carrageenan solution in order to initiate aedema of the right hindpaw. The percentage inhibition of inflammatory edema was registered at 2^{nd}, 3^{rd},4^{th} and 24^{th} hour thereafter by plethysmometer. Statistical evaluation was performed by SPSS 19.0. software.

Results: Comparison between two groups did not show differences in values of serum levels of transaminases, cholinesteraseand amylase. Results of RPM-simulation group showed significant decrease of serum concentration of creatinine ($p<0,0001$) and elevation in urea concentration($p<0,001$) compared with S-controls. Significantly decreased levels of glucose were established for RPM-simulation group compared with S-controls ($p<0,0001$). The results have shown no differences in TSH levels between two groups but decreased level in serum cortisol($p<0,0001$) and insignificant decrease of aldosterone in RPM-simulation group. Quantitative measurement of development of rat paws swelling have shown tendency for reduction (insignificant decrease) in RPM-simulation group at 2-nd hour compared with S-control, while at 3-rd and 4-th hour the aedema in RPM-simulation group undergone rise tendency and became statistically significant at 24-th hour ($p<0,05$).

Conclusion: Rats exposed to RPM-simulated microgravity have demonstrated specific time-related response to carrageenan-induced inflammation which differs to reaction of control animals. Decrease in creatinine and glucose serum levels in RPM-simulated rats does not correlate directly with aedema but can be treated in "antistress" aspect of total simulated mictogravity influence.

NEUROPROTECTIVE AND REDOX - MODULATING POTENTIAL OF COPTISINE IN ALZHEIMER'S TYPE DEMENTIA IN MICE

Elina Tsvetanova[1], Albena Alexandrova[1,2], Almira Georgieva[1], Maria Lazarova[1], Reni Kalfin[1], Diamara Uzunova[1], Luciano Saso[3], Lyubka Tancheva[1]

[1]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]National Sports Academy, Sofia, Bulgaria
[3]University of Sapienza, Rome, Italy

Background: Neurodegenerative disorders are characterized by progressive cognitive decline, which results in irreversible loss of neurons with subsequent disability. They affect millions of people worldwide and currently are incurable. One of the earlier events in these pathologies has been associated with oxidative stress induction. Some of the effective strategies in the treatment of Alzheimer's disease (AD) are focused on using some antioxidants and acetylcholinesterase inhibitors as preventive and therapeutic molecules Coptisine (Cop) is a plant alkaloid and the main constituent of various plant extracts, used for treating of Alzheimer's disease (AD) or age-related central nervous system disorders in Chinese medicine. However, little is known regarding its possible effects on neurodegenerative processes.

Objective: This study aimed to investigate the potential neuroprotective and redox-modulating potential of Cop in the brain of mice with experimental dementia of AD type.

Methods: Cop was applied in two model systems in male IRC mice, which were divided into following groups: i) In healthy animals 1) Control group (Saline, 0.1 ml/10 g, i.p); 2) Group treated with a single dose of Cop (3.5, 7 and 10mg/kg, i.p); ii) In mice with dementia 1) Control group (Saline, 0.1 ml/10 g, i.p 2) Scopolamine 1 mg/kg, 9 days 3) (Scopolamine 1 mg/kg, 9 days, i.p), treated with Cop (3.5 mg/kg, 9 days, i.p). On 1st, 24th and 48th hour as well as on 7th and 14th day after Scopolamine treatment, all groups underwent behavioral verification tests (Step-through test and Hole-board). After the tests' performance, the brains were processed for biochemical analyses – measurements of acetylcholinesterase (AChE) activity and changes in oxidative status (lipid peroxidation, glutathione level and antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase.

Results: In healthy animals Cop treatment 3,5mg/kg did not alter both memory and oxidative status, but inhibited AChE (38% vs control). In mice with Scopolamine-induced dementia, Cop treatment in dose dependent manner recovered impaired memory of demented mice, accomplished by inhibition of both AChE and oxidative stress parameters (lipid peroxidation, superoxide dismutase and glutathione peroxidase).

Conclusion: In conclusion Cop demonstrated significant redox-modulating potential, improved memory and orientation of demented mice and inhibited the activity of the acetylcholine esterase in a murine model of AD type dementia. Cop could be a promising therapeutic target that could reduce, prevent and counteract neurodegenerative incidence.

INVESTIGATIONS ON THE EFFECTS OF NATURAL SUBSTANCES COMBINATION IN ALZHEIMER'S TYPE DEMENTIA

Natali Ivanova[1], Lyubka Tancheva[2], Maria Lazarova[2], Diamara Uzunova[2], Borislav Minchev[2], Polina Kirova[2], Reni Kalfin[2]

[1]Faculty of Chemistry, South-West University "Neofit Rilski", Blagoevgrad, Bulgaria
[2]Institute of Neurobiology, Bulgarian Academy of Science, Sofia, Bulgaria

Background: One of the most important directions in modern experimental science is discovery of biologically active substances and their combinations with a potential for therapy and prevention of neurodegenerative disorders including Alzheimer's disease.

Objective: Aim of this study was to investigate memory-enhancing effects of innovative experimental combination (EC) containing seven biologically active substances in Alzheimer's type dementia (ATD) induced by scopolamine (Sco) in male Wistar rats.

Methods: Animal model of dementia in male Wistar rats was produced by 11 days of Sco (2 mg/kg, intraperitoneally) treatment. EC was applied daily orally in an effective dose for 40 days before and 11 days simultaneously with Sco (total of 51 days). Several behavioral tests were used to evaluate changes in short-term and long-term memory (Step through passive avoidance test, Novel Object Recognition test, T-maze test and Barnes maze test), followed by some histological and biochemical studies. Advanced exploratory analysis of the principle components (rotation Equamax) and additional hierarchical cluster analysis were used for calculation of the coefficient of memory restoration in each group.

Results: The behavioral studies revealed a significant memory recovery effect in EC-treated dement rats (the coefficient of memory restoration is 91 %). Statistical analyses which integrated behavioral and histological data showed

clearly distinguished groups organized in separate clusters: healthy controls, Sco-treated rats and EC-treated dement rats. Last cluster was very near to those of control healthy animals. At least three different mechanisms are involved in observed memory protective effect of EC in experimental model of ATD - antioxidant capacity, anti-inflammatory effect and neuromodulatory activity confirmed in prefrontal cortex and hippocampus, which are two brain structures mostly related to memory. Future clinical studies will reveal if this effect should be confirmed in volunteers.

Conclusions: The group of dement rats treated with EC is practically undistinguished by the healthy controls. EC is safe and effective after long term administration.

DOES MELATONIN APPLICATION AMELIORATE FRUCTOSE-INDUCED DISORDERS?

Petya Hadzhibozheva[1], Zhivka Tsokeva[1], Lilia Pashova – Stoyanova[1], Maria Ganeva[1], Anna Tolekova[2], Galina Ilieva[1], Tsvetelin Georgiev[1]

[1]Dept.of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2]Medical College, Trakia University, Stara Zagora, Bulgaria

Background: Long-term fructose consumption has been reported to induce metabolic changes which could further lead to the development of cardiovascular disease, diabetes mellitus type 2, and often are accompanied by smooth muscle organ dysfunction such as gastroparesis, constipation and lower urinary tract symptoms.

Objective: We investigated the possible protective effects of melatonin (MLT) administration on metabolic and smooth muscle disorders induced by fructose drinking in rats.

Methods: 24 mature male Wistar rats were randomly divided into four groups (n = 6): (1) control group, (2) MLT group, (3) fructose group, and (4)

fructose + MLT group. Fructose (15%) was given in drinking water. MLT was administered orally 0.3 mg/kg/three times a week. At the end of the experimental period (11 weeks), plasma levels of glucose and creatinine, as well as the lipid profile were assessed. The contractile properties of reservoir smooth muscle organs were studied using stimulation by Angiotensin II in the isolated tissue bath system.

Results: Drinking a fructose solution increased plasma glucose and creatinine levels, as well as triglycerides. The responses to Angiotensin II of the smooth muscle preparations from the fructose group were with reduced parameters when comparing to those of controls. MLT supplementation significantly lowered blood sugar and creatinine, but the triglyceride levels of fructose + MLT group remained higher. An improvement in Angiotensin II-evoked smooth muscle activity was observed in preparations from the urinary bladder and stomach. However, the responses to Angiotensin II of rectal preparations were not ameliorated.

Conclusions: The results indicate that MLT application counteracts some of the metabolic disorders caused by the chronic fructose drinking. Furthermore, MLT may be a promising pharmacological agent against fructose-induced smooth muscle dysfunction, especially by ameliorating the contractile activity of urinary bladder and stomach.

COMPARATIVE EVALUATION OF OSTEOCALCIN AND VITAMIN D SERUM LEVELS IN WOMEN WITH POSTMENOPAUSAL OSTEOPOROSIS AND HEALTHY WOMEN IN MENOPAUSE

Tatyana Simeonova[1], Krasimira Stefanova[2], Ivelina Himcheva[1], Petya Dragomirova[3], Pavlina Yordanova-Laleva[4], Sergey Kostadinov[5], Zdravka Radionova[6], Boryana Ruseva[1]

[1]Department of Physiology and Pathophysiology, Medical University-Pleven, Pleven, Bulgaria
[2]Diagnostic Consulting Center II – Pleven, Pleven, Bulgaria
[3]Department of Anatomy, Histology, Cytology and Biology, Medical University –Pleven, Pleven, Bulgaria
[4]Faculty of Pharmacy, Medical University –Pleven, Pleven, Bulgaria
[5]Department of General Medicine, Forensic Medicine and Deontology, Medical University –Pleven, Pleven, Bulgaria
[6]Faculty of Public Health, Medical University –Pleven, Pleven, Bulgaria

Background: Osteocalcin is a bone formation marker. It is used to determine the quality of bone tissue, and vitamin D is required for its formation. There is evidence that measurement of osteocalcin levels are useful for screening and diagnosis in postmenopausal osteoporosis.

Objective: Our study aimed to assess osteocalcin levels concerning vitamin D, the other parameters of calcium-phosphorus metabolism in women with postmenopausal osteoporosis and women in menopause, and the utility of its serum levels, as an epidemiological assessment tool to diagnose postmenopausal osteoporosis.

Methods: Two groups of Bulgarian menopausal women took part in the study - 26 women with osteoporosis and 22 without osteoporosis. Diagnosis was made by measuring bone mineral density of the lumbar spine. Immunological and biochemical tests on venous blood were made to measure serum levels of osteocalcin, vitamin D and alkaline phosphatase, calcium and phosphorus respectively.

Results: The mean levels of serum osteocalcin in patients were 23,41±10,08 ng/ml and 20,83±8,1 ng/ml in controls. The mean levels of vitamin D showed insufficiency in both groups, 48,09±19,41 nmol/l in patients and 48,34±23,1 nmol/l in controls. Calcium, phosphorus and alkaline phosphatase were within the referent values. There are no statistically significant differences in the values of vitamin D, osteocalcin, and parameters of calcium-phosphorus metabolism in both groups. Moderate negative correlations between serum osteocalcin levels with age ($p<0.05$) and BMI ($p<0.05$) in group with OP were found. A moderate positive correlation between osteocalcin with vitamin D levels was found ($p<0.01$), and a strong positive correlation with alkaline phosphatase ($p<0.01$) were found in the same group.

Conclusion: Our results have demonstrated average osteocalcin levels in the normal range, as interpreted according to the kits used and no significant differences between the study group and the healthy controls. We could not confirm the benefit of testing serum OC levels for screening and diagnosis of postmenopausal osteoporosis. A disadvantage of is the small number of patients studied, and also, impossibility to compare the obtained results due to the lack of published data about osteocalcin in women with postmenopausal osteoporosis in Bulgaria. To be useful in our clinical practice, the data should be confirmed by assessment of a larger number of patients.

CYTOTOXIC EFFECT OF TACRINE ON HUMAN MELANOMA CELLS A2058

Viktor Yotov[1], Yana Feodorova[2,3], Ekaterina Zaytseva[1], Danail Minchev[2,3], Marian Draganov[2], Valentin Turiyski[1]

[1]Department Medical Physics and Biophysics, Medical University of Plovdiv, Plovdiv, Bulgaria,
[2]Depsrtment Medical Biology, Medical University of Plovdiv, , Plovdiv, Bulgaria,
[3]Division of Molecular and Regenerative Medicine- Research Institute, Medical University of Plovdiv, Plovdiv, Bulgaria

Background: Tacrine is one of the first compounds used for treatment of Alzheimer's disease. It inhibits the activity of several enzymes, such as acetylcholinesterase, butyrylcholinesterase and histamine N-methyltransferase, and can perturb DNA transcription by inhibiting the activity of topoisomerases. Those features have attracted attention to tacrine and its derivatives as potential anticancer drugs.

Objective: In this study we investigated in vitro the cytotoxic activity of tacrine on human melanoma cells A2058.

Methods: Tacrine (1,9-amino-1,2,3,4-tetrahydroacridine) was used in the following concentrations: 1, 10, 20, 40, 60, 80 and 100 μg/ml. Cells were treated with tacrine for 2, 4, 24, 48 and 72 hours. At the end of each incubation period, the medium containing tacrine was exchanged by fresh DMEM growth medium. Cell vitality was measured in all samples by MTT test at the 72nd hour. Cells were inspected microscopically and images were acquired for evaluation of the cell monolayer and the morphological changes of treated and control cells that have occurred during the treatment. In an independent experiment cell vitality was determined in the real-time xCELLigence-cell analyzer. In this case, cells were treated with tacrine in concentrations from 1 to 80 μg/ml. For the calculation of the IC_{50} values from the MTT test the online calculator AAT Bioquest was used. The IC_{50} values

in the xCELLigence experiment were calculated by the integrated machine analysis software.

Results: Microscopically detectable changes of A2058 cells were observed after 2 hours of treatment with the three highest concentrations of tacrine: 60, 80 and 100 μg/ml. The monolayer was destroyed, the cells got rounded and died. The same changes became discernible in cells treated with 40 μg/ml tacrine after a 24-hour treatment, as well as in cells treated with 20 μg/ml after 72 hours. A reduction in the number of viable cells is also present for the samples treated with the lowest tacrine concentrations, 1 and 10 μg/ml, but the number of dead cells is small and the monolayer is sustained. The results of the MTT test confirm the microscopic observations. The number of viable cells treated with 20 μg/ml tacrine drops to 90.29% after 2 hours of treatment and to 50.44% after 72 hours. Tacrine in a concentration of 40 μg/ml reduces cell vitality from 73.24% (2 hours of treatment) to 4.81% (72 hours of treatment), consistent with the presence of single living cells only. The trend for increasing cell death of the treated samples correlates both with increasing tacrine concentrations and with longer treatment times.

Conclusion: Combined, the results from the MTT test, the microscopic analysis and the xCELLigence system show a strong time-and-dose-dependent cytotoxic effect of tacrine on human melanoma A2058 cells.

ULTRASONIC VOCALIZATIONS MEASURING AFFECTS IN RODENT MODELS OF AFFECTIVE DISTURBANCES

Andrey Popatanasov, Lyubka Tancheva, Maria Papazova, Reni Kalfin
Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Communication has paramount importance in the life of both humans and mammals and is one of the best ways to share information, emotions, emotional states, etc. Social interactions and behavior in great extent depend on the emotions which have a significant neurological basis. Rodents are one of the most commonly used laboratory animals used to model human disease, however, a significant part of their vocal communication lies outside the range of human hearing (< 20 kHz), i.e. in the ultrasound range (> 20 kHz). This makes it difficult to study the fuller spectrum of their communication and therefore is a rarely researched area. Affective disorders are an important component of many human behavioral and neurological disorders, howbeit due to ethical and other reasons often rodents are used for their study instead humans. Unfortunately, common methods of research on affective disorders tend to exclude the key communication dimension, which can make the results obtained ambivalent or incomplete.

Objective: Aim of the present study was to investigate ultrasonic vocalization in rodent model of affective disturbances.

Methods: For the study were used male Wistar rats. Model of non-motor symptoms of Parkinson's disease was developed with 6-hydroxydopamine. Ultrasound vocalizations were recorded during the behavioral tests and analyzed afterwards.

Results: The results show that there is different pattern of ultrasonic vocalizations expression in the studied model compared to the controls.

Conclusions: Ultrasound vocalizations present a useful tool to study the affective disturbances in various pathologies in rodents, which necessitate further studies and explorations.

FACTORS ASSOCIATED WITH INCREASED RISK OF DEPRESSION IN INTERNATIONAL STUDENTS

Gabriela Panayotova[1], Silviya Nikolova[2], Dobrinka Doncheva[1], Zlatislav Stoyanov[1], Margarita Velikova[1]

[1]Department of Physiology and Pathophysiology, Medical Faculty, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria
[2]Department of Social Medicine and Health Care Organization, Faculty of Public Health, Medical University"Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria

Background: Medical students experience high levels of stress during their course of academic studies.

Objective: Our survey aimed to examine the sociodemographic, study-related, and lifestyle factors that affect international students' mental health and increase the risk of developing depression.

Methods: The participants – 1st and 2nd-year medical students filled Beck Depression Inventory (BDI), University Stress Scale (USS), and Perceived Stress Scale (PSS). 20.1% of the students showed high BDI scores, corresponding to different degrees of depression.

Results: The results revealed a correlation between depression, academic stress, and perceived stress. The rate of depression among medical students does not appear to be a result of the medical school experience alone. We find a complex combination of factors that may influence the nature of depression among medical students predisposed to it, such as gender, self-evaluated financial status, and exercise frequency.

Conclusions: According to the sociodemographic factors, our research shows that depression was more explicitly associated with females as well as the overall perception of being unhealthy or sick.

A SURVEY ON THE MEDICAL STUDENTS' OPINION ABOUT THE EFFECTIVENESS OF E-LEARNING IN A PHYSIOLOGY COURSE

Antonia Hachmeriyan, Irina Pashalieva, Zlatislav Stoyanov, Margarita Velikova

Department of Physiology and Pathophysiology,
Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria

Background: Learning in an *electronic environment* (*e-learning) affects students'* motivation, knowledge acquisition, and academic achievement.

Objective: To study the opinion of medical students in the Bulgarian-language and English-language programmes about the effectiveness of e-learning in the physiology course.

Methods: An anonymous survey was conducted among 227 second-year Bulgarian (BS) and international (IS) medical students.

Results: Almost all students aim to acquire thorough knowledge (100% of BS and 97.5% of IS). Largest number of students state that: physiology facilitates their studies in other subjects (88.78% - BS; 48.33% - IS); prefer face-to-face training (86% - BS; 53.33% - IS); the electronic environment makes more difficult studying physiology (77.57% - BS; 52.5% - IS); they lose motivation in an electronic environment (54.21% - BS; 43.33% - IS); classes in an electronic and face-to-face environment do not provide the same opportunity to acquire knowledge (78.50% - BS; 51.67% - IS); the class discussions contribute most to understanding the course topics (45.79% - BS; 40.00% - IS). Concerning the practical classes, BS prefer group tasks (49%), while IS evaluate discussions and group tasks equally (43.33%). The highest percentage of BS believe the advantage of e-learning is that it saves time (82.24%), while IS prefer the possibility of joining from different location (56.67%). As a most objective type of examination for assessing students' knowledge BS consider the hybrid form (test and oral exam)(40.19%), while

IS prefer the test (59.17%).

Conclusions: A disapproval of e-learning is clearly expressed and more pronounced among Bulgarian medical students (0.9% - BS; 5% - IS), while most students prefer in person classes (86% - BS; 53.33% - IS). IS from the English-language program find less challenging the e-learning as far as many approve of the hybrid form of education (43.33%, vs. 13.08% BS) and think that online and in person classes provide the same opportunity to acquire knowledge (25.83% - IS; 10.28% - BS).

GLOBAL MOTION DISCRIMINATION IN THREE DEVELOPMENTAL DISORDERS: ASD, ADHD AND DD

Nadejda Bocheva, Ivan Hristov, Simeon Stefanov, Tsvetalin Totev, Milena Mihaylova

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Developmental disorders such as Autism Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD), and Developmental Dyslexia (DD) have different diagnostic defining features. However, they share some common deficits like more visual problems, atypical eye movements and reduced perceptual efficiency.

Objective: The aim of the present study was to compare the ability to discriminate global motion direction, and the eye movements of children and adolescents with ASD, ADHD, DD, or typical development (TD).

Methods: One hundred and eleven children and adolescents from four groups: with ASD, ADHD, DD, or TD had to define the average direction of movement of 30 Laplacian-of-Gaussian micro-patterns. The directions of the moving micro-patterns were determined by a normal distribution with a standard deviation of 2°, 5°, 10°, 15°, 25°, and 35°corresponding to the

external noise level. Simultaneously, the eye movements of the participants from all the groups were recorded and analyzed later.

Results: The data obtained showed that global motion direction discrimination thresholds increase on increasing external noise for all participants. TD group showed the least individual differences in motion direction thresholds compared to all other groups. The external noise affected motion direction thresholds in the ASD and DD groups to a larger extent than in the TD and ADHD groups. The global motion direction discrimination thresholds were higher for ASD and DD participants at all noise levels. Equivalent-noise analysis revealed a tendency for the group with TD for a higher ability to integrate local motion direction. The results of the group with ADHD were very similar to the control group results. However, groups with ASD and DD showed lower integration ability and diminished sensitivity to global motion direction, not resulting from the more imprecise determination of local motion directions of the micro-patterns but from diminished averaging efficiency. Eye-tracking results showed a significantly larger number of fixations with diminished duration for the group with ASD compared to the control group in high external noise, which suggests greater fixation instability in conditions of greater uncertainty.

Conclusions: The results obtained imply lower sensitivity to mean motion direction in ASD and DD compared to TD, especially in high external noise, suggesting that participants with ASD and DD have a poor ability to integrate the local motion information in low-density displays.

3D BIOPRINTING – A MODERN APPROACH TO ONCOLOGY DISEASES

Viktor Velyanov, Kamelia Bratoeva

Department of Physiology and Pathophysiology, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria

Background The major difficulty in cancer research is to simulate the complex relationship between the tumor cells, microenvironment and host immune cells. This creates a challenge in developing models for cancer disease and drug testing. The existing experimental models *(in vitro and in vivo)* have their advantages, however they are unable to predict drug effects or disease outcomes, due to lack of physiological relevance.

Objective: The aim of this study was to analyze the qualities of the modern experimental models for cancer research available in the published literature and the role of 3D bioprinting in cancer experiments.

Methods: A research was conducted in the electronic database of PubMed, ResearchGate, ScienceDirect and Google scholar. This review does not comprehensively cover the literature however; it highlights issues confronted in the field.

Results: The available models for studying cancer are numerous and diverse in their principles. Two dimensional models fail to provide the necessary environment and complexity of tumor physiology progression and metastasis. Animal models are slow, costly and limited by ethical reasons, besides there are disparities between animal and human models of disease. Recently some 3D models, such as the spheroids and organoids were developed onto different scaffolds and cancer-on-chip devises, unfortunately even they cannot mimic the close relationship between different cells types and the complexity of cancer architecture. It can be said that 3D bioprinting is superior to all these methods. It can produce multicellular constructs, cancer stroma and even

blood vessels, thus resolving many of the older methods' issues. Bioprinting can also create special 3D models that could capture the key characteristics of cancer cells and their microenvironment. Published researches show remarkable results in the fields of cancer surgery, chemotherapy drug screening, metastasis pathophysiology and diagnosis.

Conclusions: 3dimensional bioprinting allows for the creation of complex bio structures that are able to interact with each other. This allows the recreation of living neoplastic tissues that closely resemble the real ones. They can be used for better understanding of cancer pathophysiology and personalized treatment compared to other methods. However further studies are required so that many aspects of the process can be improved like high resolution printing, tissue stability, sterility and cost.

CHANGES IN SERUM LEVELS OF MATRIX METALLOPROTEINASE-1 AND TISSUE INHIBITOR OF METALLOPROTEINASES-1 IN PATIENTS WITH ESSENTIAL HYPERTENSION

Krasimir Kostov[1], Ademira Borisova[1], Alexander Blazhev[2]

[1]Department of Pathophysiology,
Medical University-Pleven, Pleven, Bulgaria
[2]Department of Biology, Medical University-Pleven, Pleven, Bulgaria

Background: Hypertension (HTN) is a leading risk factor for cardiovascular (CV) disease. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are thought to be actively involved in the remodeling of the CV extracellular matrix (ECM) during hypertensive damage.

Objective Therefore, in this study, we aimed to investigate serum levels of MMP-1 and TIMP-1 in patients with essential HTN and compare them with those of normotensive individuals.

Methods: We measured serum concentrations of MMP-1 and TIMP-1 in 60

patients with HTN and 20 healthy controls using an ELISA.

Results: The obtained results showed that in patients with HTN, the mean levels of MMP-1 (1.82 ± 0.9 ng/mL) were significantly higher ($p = 0.03$) than the mean levels in the control group (1.19 ± 0.7 ng/mL). The levels of TIMP-1 in patients with essential HTN (0.44 ± 0.1 ng/mL) were also significantly higher ($p = 0.005$) than those in the control group (0.33 ± 0.1 ng/mL).

Conclusions: In HTN, elevated serum MMP-1 levels may be associated with increased collagen degradation in the CV ECM, whereas elevated TIMP-1 levels may favor its accumulation and the development of pathological remodeling and fibrosis of the heart and arterial vessels.

ROLE OF NOCICEPTIN AND ANALOGUES IN THE INTERACTION BETWEEN NITRICOXIDERGIC AND ENDOCANNABINOIDERGIC SYSTEMS AFTER CHRONIC IMMOBILIZATION STRESS IN RATS

Ivelina Himcheva[1], Galya Stavreva[1a], Adriana Bocheva[1]

[1]Department of Physiology and Pathophysiology, Faculty of Medicine, Medical University of Pleven, Pleven, Bulgaria
[2] Department of Pharmacology and Toxicology, Medical University – Pleven, Pleven, Bulgaria

Background: Stress is known to elicit pain alleviation, a phenomenon referred to as stress-induced analgesia (SIA). Two forms of SIA are commonly distinguished: an opioid-mediated and a non-opioid one. The non-opioid one comprises several neurotransmitter systems. Both the nitricoxidergic and endocannabinoidergic (ECS) systems participate in the descending antinociceptive system of the body. Nitric oxide (NO) is a unique neurotransmitter, which participates in many physiological and pathological processes in the organism. NO plays an important role in the initiation and maintenance of pain. The ECS has a well-established role in the modulation of

pain perception and behavioral responses after stress. Nociceptin/ Orphanin FQ(N/OFQ) and analogues are neuropeptides, and neuromodulators, which are able to inhibit the expression of some forms of SIA. N/OFQ is a heptadecapeptide that has been found to play a role in pain perception.

Objective: This study aimed to investigate the effects of interaction between the ECS and nitricoxidergic systems with the novel analogues of nociceptin N/OFQ(1-13)NH_2, on nociception after chronic immobilization stress (CIS).

Methods: The experiments were carried out on male Wistar rats. For CIS the animals were placed in a tube for 3 hours daily for 4 days. Analgesic effects were examined by Paw pressure (Randall-Selitto) test. All novel analogues of N/OFQ were injected intraperitoneally (i.p) with different combinations of cannabinoid receptor type 1 (CB1) agonist anandamide (AEA) or antagonist (AM251) along with NO-precursor (L-arginine) or inhibitor (L-NAME) of the NO-synthase (NOS). Statistical analysis was performed using one-way ANOVA.

Results: The results showed that L-arginine administration along with CB1 agonist (AEA) immediately after the end of stress led to a tendency to increase immobilization SIA, while the combination of L-arginine with CB1 antagonist (AM251) significantly decreased the pain threshold compared to a group that underwent chronic stress only. Application of both L-NAME along with AEA and L-NAME with AM251 after the end of CIS significantly reduced the pain threshold compared to a group that underwent chronic stress only. Nociceptin and analogues administered with different combinations of L-arginine or L-NAME, along with AEA or AM251 after the end of stress decreased the pain threshold significantly compared to a group with CIS only.

Conclusions: Our experiments confirmed that the nitricoxidergic and endocannabinoidergic systems interact between them in the modulation of immobilization SIA and participated in the analgesic effects of nociceptin and analogues after CIS.

RELATIONSHIPS OF COPPER AND SELENIUM SERUM CONCENTRATIONS TO HEMATOLOGIC PARAMETERS IN PREGNANT WOMEN HAVING LOW-RISK AND HIGH-RISK PREGNANCY

Tsvetelina Petkova-Marinova[1], Boryana Ruseva[1], Bozhanka Paneva-Barzashka[2], Bisera Atanasova[3], Pavlina Laleva[4]

[1]Department of Physiology and Pathophysiology, Faculty of Medicine, Medical University – Pleven, Pleven, Bulgaria
[2]Medical Center "Clinical Institute for Reproductive Medicine St. Elisaveta", Pleven, Bulgaria
[3]University Hospital "Alexandrovska", Department of Clinical Laboratory, Faculty of Medicine, Medical University, Sofia, Bulgaria
[4]Department of Medical Physics, Biophysics, Pre-clinical and Clinical Sciences, Faculty of Pharmacy, Medical University – Pleven, Pleven, Bulgaria

Background: Pregnancy period is characterized with increased iron (Fe) necessities due to the expansion in maternal erythrocyte mass and intensive tissue growth. The Fe homeostasis is closely connected with the regulation of other trace elements as copper (Cu) and selenium (Se). Their interactions and their binding inter-links are the focus of extensive studies.

Objective: The objective of this study was to survey the relationships of Cu and Se serum concentrations to hematologic parameters in pregnant women having low-risk and high-risk pregnancy.

Methods: Forty (40) pregnant women having low-risk (n=20) and high-risk pregnancy (n=20) were studied in the beginning of second and third trimester. The high-risk pregnancies were preceded by reproductive failures and/or associated with autoimmune and chronic inflammatory disorders – Hashimoto thyroiditis and obesity. We determined the Cu and Se serum concentrations, the red blood cell count, hemoglobin concentration, hematocrit, erythrocyte indices (MCV, MCH and MCHC), and the biochemical markers of Fe metabolism serum ferritin, serum Fe and total Fe-binding capacity, with subsequent calculation of transferrin saturation.

Results: Our results demonstrate a statistically significant higher proportion of pregnant women having below-threshold serum Se concentrations in the high-risk pregnancy group compared to the control group in the beginning of second trimester ($p=0.006$). We found a statistically significant positive correlation between the serum Se concentrations and hematologic indicator MCV in the studied women with low-risk pregnancy for the second-trimester samples ($r=0.657$; $p=0.039$); as well as a statistically significant positive correlation between the serum Cu concentrations and MCH in the women with high-risk pregnancy for the third-trimester samples ($r=0.805$; $p=0.029$). **Conclusions:** Trace elements Cu and Se play significant roles in the erythropoiesis process during low- and high-risk pregnancy. The clinic studies investigating the Cu and Se participation in the Fe homeostasis maintenance during pregnancy should be extended. This would allow optimization of the intake and application term of the trace elements Cu and Se in pregnant women having low-risk and high-risk pregnancy.

EFFECTS OF NOCICEPTIN AND ANALOGUES ON THE CANNABINOIDERGIC SYSTEM ON PAIN AFTER CHRONIC IMMOBILIZATION STRESS

Ivelina Himcheva[1], Galya Stavreva[2], A. Borisova[1], Armine Grigorian[1], Aneliya Dimitrova[1], Emilia Naydenova[3], Adriana Bocheva[1]

[1] Department of Physiology and Pathophysiology, Faculty of Medicine, Medical University of Pleven, Pleven, Bulgaria
[2] Department of Pharmacology and Toxicology, Medical University of Pleven, Pleven, Bulgaria
[3] Department of Organic Chemistry, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

Background: Stress provokes stress-induced analgesia (SIA), which depends on an opioid and non-opioid components. The non-opioid one comprises several systems participating in the descending antinociceptive

system of the body: cannabinoidergic, adrenergic and nitricoxidergic, which alter pain perception and modulation of behavioral responses after stress. The endocannabinoid system (ECS) has a proven role in the modulation of pain perception at both the central and peripheral levels. Nociceptin/Orphanin FQ(N/OFQ) is a heptadecapeptide that has been found to play a role in pain perception.

Objective: This study aimed to investigate the effects of novel nociceptin N/OFQ(1-13)NH_2 analogues on nociception after chronic immobilization stress (CIS) and the involvement of the ECS in analgesic effects.

Methods: The experiments were carried out on male Wistar rats (180-200g). Analgesic effects were examined by paw-pressure (PP) test. For CIS the animals were placed in a tube for 3 hours daily for 4 days. Nociceptin analogues were synthesized in the laboratory of Prof. Naydenova at the University of Chemical Technology and Metallurgy – Sofia. All novel analogues of N/OFQ were injected at a dose of 10 μg/kg, anandamide (CB1 agonist at a dose of 1mg/kg) and AM251 (CB1 antagonist at a dose of 1,25 mg/kg) were injected intraperitoneally (i.p). Statistical analysis was performed using one-way ANOVA.

Results: The results showed that CIS caused an increase in pain threshold, which was not statistically significant versus the controls. Nociceptin and analogues administered after CIS decreased the pain threshold significantly compared to a group that underwent chronic stress only. The administration of anandamide led to a significantly increased pain threshold, while AM251 after CIS significantly decreased the pain threshold compared to a group that underwent chronic stress only. Nociceptin and analogues co-administered whit anandamide and AM251 significantly decreased the pain threshold compared to the CIS.

Conclusions: The results suggest the participation of endocannabinoidergic system in the analgesic effects of nociceptin and analogues after chronic immobilization stress.

IS UNDERCARBOXYLATED MGP A POTENTIAL MARKER FOR VITAMIN K2 STATUS OR VASCULAR CALCIFICATION IN PATIENTS WITH CKD?

Borislav Ignatov[1], Tsvetelina Eftimova[2], Tatyana Simeonova[1], Petya Dragomirova[3], Pavlina Yordanova-Laleva[4], Sergey Kostadinov[5], Aneliya Dimitrova[1]

[1]Department of Physiology and Pathophysiology, Medical University of Pleven, Pleven, Bulgaria
[2] Department of Dialysis, MHAT "Cherven bryag", Cherven bryag, Bulgaria
[3]Department of Anatomy, Histology, Cytology and Biology, Medical University of Pleven, Pleven, Bulgaria
[4]Faculty of Pharmacy, Medical University of Pleven, Pleven, Bulgaria
[5]Department of General Medicine, Forensic Medicine and Deontology, Medical University of Pleven, Pleven, Bulgaria

Background: The role of vitamin K2 in cardiovascular health is now considered indisputable. In recent years, data about vitamin K2 deficiency in healthy individuals and those suffering from various diseases have been accumulated. Recent studies show poor vitamin K2 status in CKD patients. The ucMGP is considered to be a biomarker of vitamin K2 status.

Objective: To assess the levels of ucMGP in patients with CKD and its role as a biomarker for vitamin K2 deficiency.

Methods: 67 adult patients aged between 31-87 years, mean age 63.97±12.77 years, took part in the study. They were divided into groups according of glomerular filtration rate: group I (GFR ≥ 90 ml/min), group II (GFR - 89-45 ml/min), group III (GFR - 44-15 ml/min). Venous blood were tested for calcium, phosphate and creatinine using biochemical analyzer Cobas E311, Roche Diagnostic. UcMGP concentrations were measured with the Human Undercarboxylated Matrix Gla Protein ELISA Kit, Abbexa, UK.

Results: Serum concentrations of calcium and phosphate were in the reference ranges of the commercial kits used in all three groups. There was

a gradual increase in the concentrations of the calcium with the decrease of glomerular filtration. The differences between groups are not statistically significant. Phosphate levels showed progressive increase, and we found statistical significant difference between groups I and III (*p* < 0.05). The levels of ucMGP were 2.08±0.83ng/ml in group I, 1.98±0.47ng/ml in group II, and 1.78±0.64ng/ml in group III. Concentration of ucMGP below 1ng/ml to undetectable is considered normal in healthy adults for Elisa Kit used in our study. We found higher circulating levels in all three studied groups. The mean levels of ucMGP showed a gradual decrease from group I to III. The differences are not statistically significant. No significant correlations were observed between ucMGP levels and any demographic or biochemical characteristic in the three groups of patients. The percentage of patients with cardiovascular disease increases in the groups with decrease of GFR. We found statistically significant difference between group I and II – t(46)=2.333, p=0.024, and group I and III – t(43)=3.745, p=0.001, but not between group II and III – t(39) = 1.185, p=0.243.

Conclusions: Undercarboxylated MGP appears to be a good marker for vascular calcification and increased cardiovascular risk and results of our study are consistent. However, its application in clinical practice is still hampered by the lack of widely available commercial assays and therefore the lack of standardized reference values for easy comparison of the obtained data. The benefit of using ucMGP as a marker for vitamin K2 status remains debatable. Our data suggest poor vitamin K2 status, but more data from studies in larger groups are needed before they can be confirmed.

EFFECTS OF NOCICEPTIN AND ANALOGUES AND THE INVOLVEMENT OF THE NITRICOXIDERGIC SYSTEM ON NOCICEPTION AFTER CHRONIC IMMOBILIZATION STRESS

Ivelina Himcheva[1], Galya Stavreva[2], Armine Grigorian[1], Aneliya Dimitrova[1], Emilia Naydenova[3], Adriana Bocheva[1]

[1] Department of Physiology and Pathophysiology, Faculty of Medicine, Medical University of Pleven, Pleven, Bulgaria
[2] Department of Pharmacology and Toxicology, Medical University of Pleven, Pleven,Bulgaria
[3] Department of Organic Chemistry, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

Background: Stress is known to exert an influence on neuroendocrine, autonomic, hormonal, and immune functioning. Various stress models have been reported to induce analgesia. This is a phenomenon, referred to as stress-induced analgesia (SIA). One of the mechanisms known to play a part in the response of an organism to stress is the activation of the endogenous opioid system. Endogenous opioid peptides take part in various functions as hormones or neuromodulators. Nociceptin and analogues are neuropeptides, and neuromodulators, which are able to inhibit the expression of some forms of SIA. Nociceptin/Orphanin FQ(N/OFQ) is a heptadecapeptide that has been found to play a direct role in pain perception. Nitric oxide (NO) is a unique neurotransmitter, which participates in many physiological and pathological processes in the organism. NO plays an important role in the initiation and maintenance of pain.

Objective: The aim of the present study was to investigate the effects of novel analogues of N/OFQ(1-13)NH_2, where Lysine (Lys) at position 9 and/or 13 was substituted by L-ornithine (Orn) on nociception after chronic immobilization stress (CIS) and the involvement of the nitricoxidergic systems in these effects.

Methods: The experiments were carried out on male Wistar rats. For CIS the animals were placed in a tube for 3 hours daily for 4 days. Analgesic

activity was examined by nociceptive test Paw-pressure (PP). All novel analogues of N/OFQ were injected at a dose of 10 μg/kg, N^G-nitro-L-arginine methylester (L-NAME, 10 mg/kg) and L-arginine (L-arg, 1mg/kg). All drugs were dissolved in saline and were injected intraperitoneally (i.p.). Statistical analysis was performed using one-way ANOVA.

Results: Our results showed that nociceptin and analogues decreased pain threshold after CIS, which is most pronounced for [Orn^9, Orn^{13}]N/OFQ(1-13) NH_2. Co-administration of the peptides with N^G-nitro-L-arginine methylester (L-NAME) and L-arginine showed a statistically significant decrease in the pain threshold.

Conclusions: In conclusion, we suggest that in the analgesic effects of the novel analogues of nociceptin was involved nitricoxidergic system after CIS.

HELIX ASPERSA MUCUS EXTRACT WITH PROTECTIVE EFFECT ON ETHANOL INDUCED GASTRIC ULCERS IN MICE

Alexander Dolashki[1], Lubomir Petrov[2], Albena Alexandrova[2,3], Lyudmila Velkova[1], Elina Tsvetanova[3] and Pavlina Dolashka[1]

[1]Institute of Organic Chemistry with Center for Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria.
[2]National Sports Academy "Vassil Levski", Sofia, Bulgaria
[3]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Objective: This study aimed to investigate the protective effect of Helix aspersa mucus extract (ME) on ethanol induced gastric ulcers (GU) in mice and elucidate its efficacy mechanism.

Methods: The composition of the ME was studied on 12% SDS-PAGE and by mass spectrometry. Male Albino mice were divided into Control, Ethanol and Mucus+Ethanol treated groups. The gastric ulcers was induced by administration of 96% ethanol (10 ml/kg, PO). The mice of Mucus+Ethanol group were pre-

treated with ME (20 mg/kg, PO) one hour before ulcer induction.

Results: Results showed that the ME is complex mixture from antimicrobial peptides, and different proteins with antimicrobial, antioxidant and regenerative properties. Moreover, we detected that ME inhibits the growth of bacterium *Helicobacter pylori*. In the group with pre-administration of ME were observed: a small number of hemorrhagic fields; significantly reduced GU index compared to ethanol group (4.7% vs 17.3% respectively); calculated highly protection 73%; significant recovery of glutathione concentration level and activity of glutathione reductase; rise in the activity of the other antioxidant enzymes.

Conclusions: The protective effect of ME in this model of gastric injury is due to synergistic effect of different mucus compounds with antibacterial, antioxidant and regenerative properties. These studies served to create the nutritional supplement - effective in various forms of gastric injury.

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EFFECTS OF DIFFERENT LOADING ON THE POSTURAL BEHAVIOUR OF PRIMARY SCHOOL-AGE CHILDREN DURING QUIET UPRIGHT STANCE

Dragomir Ivanov, YoannaYordanova, Katerina Kirilova, Maya Ruteva, Georgi Georgiev, Katerina Stambolieva

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Existing evidence suggests a link between the weight of school backpacks and the damage that their excessive load causes. It has been found that the weight of the load should not exceed 15% of the total body weight of

the child carrying it. Carrying heavy schoolbags on daily basis in the long term leads to spinal distortions and musculoskeletal disorders. To compensate for the weight, the body reacts with postural changes, which in many cases lead to slouching and shifting the center of gravity of the body. In childhood, this overload can lead to both temporary and chronic musculoskeletal problems and pain.

Objective: The aim of this study is to evaluate the effects of symmetrical loading of the spine and shoulders on the maintenance of static postural stability in healthy primary school-age children.

Methods: Eight children (age 8-11 years) without any musculoskeletal disorders were investigated. Displacements of the center of foot pressure (COP) were recorded by a pedobarographic system during quiet stance for 30s. on firm support in four experimental conditions: unloaded stance with open eyes and with a backpack loaded with 10%, 15% and 20% of the child's bodyweight. Photos of the children in profile were taken in all experimental conditions. Postural stability was evaluated by the parameters: total length of the COP displacements (sway path) and mean sway amplitudes in the anterior-posterior and medial-lateral directions. Photogrammetry was applied for evaluation of the angles characterizing the positions of head and body ("C7-malleolus-mentum", "vertical-malleolus-auris", "acromion-mentum-horizontal").

Results: The parameter sway path did not change significantly except loading with 20% of child's bodyweight compared with the condition without loading. These changes are mainly due to increase of the mean amplitudes of postural sway in the medial-lateral direction. The photogrammetric analysis of static stance showed no significant changes of the angle "C7-malleolus-mentum" while the angles "acromion-mentum-horizontal" and "vertical-malleolus-auris" significantly increased in the three loading conditions, mainly in the 20% loading. That shows that the weight helps the body to

move the acromion backward instead of moving the mentum forward and also increases the dorsiflexion of the ankles.

Conclusion: The results suggest that stance with a bag on both shoulders with 10-20% loading affects the postural behavior by changing the static upright stance, mostly prominent when the load is 20%. The body uses ankle strategy for keeping postural stability rather than compensation with the head and upper trunk.

THE RELATIONSHIP BETWEEN SPORT PRACTICE LEVEL AND POSTURAL STABILITY IN QUIET UPRIGHT STANCE IN BOXERS

Tervel Pulev[1,2], YoannaYordanova[3], Katerina Kirilova[3], Maya Ruteva[3], Georgi Georgiev[3]. Katerina Stambolieva[3]

National Sports Academy "Vassil Levski", Sofia, Bulgaria
[2]Sport Center "Pulev", Sofia, Bulgaria
[3]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Box is a sport that requires constant attention, fast reactions, muscle force, but also intricate leg, trunk and arm coordination, body readiness and a stable posture as a basis of all subsequent movements.

Objective: The aim of this study was to evaluate the effects of sport practice level on the postural stability of boxers during quiet upright stance.

Methods: Twenty participants took part in the investigation. They were divided in three groups according to their experience in practicing box: 1-3 years, 5-7 years and more than 10 years. Postural sway was measured by a static posturographic system during quiet stance on firm support in two experimental conditions: stance with open eyes and stance with closed eyes. The duration of each trial was 30s. The parameters mean amplitudes and mean velocities of the postural sway in both directions: medial-lateral and anterior-posterior, were used for evaluation of the changes in postural stability.

Results: The 1-3 years group demonstrated higher mean amplitudes of sway in the anterior-posterior direction than the medial-lateral one, similar to untrained subjects, while in the other two groups the sway amplitudes in both directions were alike. Mean postural sway velocities in both directions increased with sport experience, as the highest velocities were observed in the most experienced group. In the first two groups the exclusion of visual information affected mostly the mean amplitudes of postural sway, mainly in the medial-lateral direction. However, this was not observed in the most experienced group that showed lesser dependence of the postural stability on the visual input. The postural sway velocities in both directions slightly increased with eyes closed, but these changes were relatively the same in all the groups.

Conclusion: Long term boxing practice could change the pattern of maintaining upright posture with low amplitude and high velocity body oscillations, similar in both directions. An interesting finding is the low effect of the visual input on the balance maintenance, which correlates with the sport experience.

EFFECT OF HIGH-ALTITUDE TRAINING ON HEMATOLOGICAL AND OXIDATIVE STRESS PARAMETERS IN ROWERS

Lubomir Petrov, Albena Alexandrova, Svilen. Neykov, Vihren Bachev

National Sports Academy"Vassil Levski", Sofia, Bulgaria

Background: The experimental research on the effects of the training model "living high, training high" required measurement of three groups of indicators: training intensity, changes in the biological indicators, and degree of hypoxia.

Objective: The research aimed to assess the effectiveness of a complex model of experimental procedures in the natural conditions of altitude

training for elite rowers.

Methods: Sixteen athletes from the men's Bulgarian national rowing team, with an average age of 18.7 years, took part in the study. They completed a three-week training camp at 2100 m above sea level at the Belmeken National Sports Base. Seven days before (T1), at the end (T2), and on the 7th (T3) and 18th day (T4) after the training camp, each participant performed an incremental test – 4 stages x 40W apart x 3min with 30 sec rest periods on a rowing ergometer. Before tests, venous blood samples were obtained for analysis of: hemoglobin concentration (Hb), the red blood cells count (RBC), hematocrit (Hct), reticulocytes (Ret‰), white blood cells (WBC), platelets (Plt), iron, total iron binding capacity (TIBC), and total plasma protein (PP).

Results: The Hb increased significantly with 8.5 g/L as well as Hct with 0.03 and RBC (with 0.39 T/L at the end of the high-altitude training camp (T2). In addition, the Hb-independent parameters PP and Plt also increased significantly in T2. However, one week after training camp (T3), all of these parameters returned to their initial levels and remained unchanged even one week later (T4). The obtained results can be explained by the onset of hemoconcentration during the high-altitude sports camp and the recovery of plasma volume up to one week after the athletes return to low altitude. We have proposed the equation for resolving the problem with the measurement of real hemoglobin changes during the "living high, training high" training model.

Conclusions: Permanent positive changes in hemoglobin and erythrocyte mass levels can be obtained with a duration of high-altitude sports camps of more than three weeks, probably at least several times a year.

STATISTICAL LEARNING AND ITS LINK TO VARIOUS COGNITIVE FUNCTIONS

Ivan Hristov, Nadejda Bocheva

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Statistical learning (SL) is a fundamental experience-independent learning process and part of a cognitive system in which regularities of our environment across space and time can be detected and extracted. It is intrinsic for infants, adults, and animals and engages various sensory modalities. Since vision is the primary source of information about the environment, SL is tightly interconnected with all general mechanisms and levels of complexity involved in visual perception and cognition. An important characteristic appropriate for testing the processes involved in SL is finding and recognizing shapes and their relative spatial disposition and, as a logical consequence, their categorization.

Objective: The present study aims to examine psychophysically the human ability to find configurations of patterns that distinguish images as same or different in visual statistical learning (VSL).

Methods: In the experiment, ten subjects were tested, aged 31 to 59 (mean age of 44). The stimuli were generated with MATLAB 2018 and presented by Psychtoolbox software on a 15.6' HP screen with 1920x1080 resolution at 60 Hz. The participants sat 50 cm in front of the screen where a pair of squared images was presented. Each pair could contain an identical configuration of red squares, or the configuration of squares only partially coincided. The images were shown in three different orientations to one another: 90°, 180°, and 270°. The participants were asked to answer with the left or right mouse button whether the images were the same or different, respectively. The stimulus duration was 3 seconds, followed by 0.5-sec interstimulus interval. The experimental session consisted of 186 trails and lasted ~ 20 min. Each

participant took part in three experimental sessions.

Results: We used two parameters – the percentage of correct responses (CR) and the reaction time (RT) needed for every answer to analyze the data. Depending on stimulus configuration, we classified the correct answers in three categories: 1/ same, when the pair of stimuli were the same; 2/ different - only one cluster of squares in the two images coincided; 3/ shifted –the images contained two identical groups of squares but in different spatial disposition. The obtained results show gradual elevation of CR through the experimental session for all types of stimuli. The accuracy for the shifted stimuli is considerably lower than the other two types, whose values reveal a close similarity. Contrary to CR, the RT decreases steeply from the beginning to the end of the trial sequence. The average participant responds faster when the stimuli are different, followed by the shifted type of stimuli, than the equal one.

Conclusions: The two tested parameters, CR and RT, unequivocally show the presence of statistical learning processes in a task consisting of an unfamiliar artificially generated configuration of elements. The results reveal that different intrinsic strategies and various probable underlying neurophysiological mechanisms participate in them.

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CONTRAST EFFECTIVENESS ELIMINATION IN THE BLUE-YELLOW AREA IN PATIENTS WITH HYPOTHYROIDISM AFTER TREATMENT WITH LEVOTHYROXINE – SIMPLE REACTION TIME STUDY

Kalina Racheva[1], Margarita Zlatkova[1,3], Tsvetalin Totev[1], Nadejda Bocheva[1], Emil Natchev[2], Roger S. Anderson[3]

[1]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Department of Endocrinology, Medical University, Sofia, Bulgaria*
[3]*School of Biomedical Sciences, Ulster University, UK*

Background: Hypothyroidism is characterized by a slowdown of all processes in the body including the visual system. Demyelination of axons in the central nervous system is observed which would also affect the optic nerve. It is known that reaction time (RT) for achromatic stimuli and the latency of cerebral VEP are prolonged in patients with hypothyroidism compared with euthyroid individuals. Hypothyroidism also affects colour vision in mice by affecting opsin production. Data in the literature indicate that visual deficits due to hypothyroidism may be reversible after treatment with levothyroxine. Previously, we measured simple reaction time (RT) to colour stimuli in the retinal periphery in untreated hypothyroid patients and controls at three contrast levels above visual threshold. Results showed prolongation of RT for patients under all conditions mostly for low contrasts, and for yellow and blue stimuli.

Objective: The aim of the present study was to determine whether the differences in RT between the hypothyroid and control groups would be eliminated after treatment with synthetic thyroxine and achieving biochemical euthyroidism.

Methods: The experiment was repeated with 14 of the patients and 23 of the controls with normal colour vision, from a total of 23 patients and 26 controls in the first study. We measured simple RT for isoluminant colour stimuli

spots, 4º in diameter, presented at 20º eccentricity along the temporal retinal meridian at three contrasts – 2.5, 4 and 8 times above the visual threshold. The chromaticity was determined using modulation from an achromatic background to to 90º, 280º, 0º and 180º in the DKL space, loosely called blue, yellow, red and green.

Results: No statistically significant difference was found between RT of patients and controls. This was demonstrated by the lack of a statistically significant effect of the group factor on RT for each colour (Blue: $p>0.52$; Yellow: $p>0.96$; Red: $p>0.25$; Green: $p>0.27$). The contrast dependence of RT is preserved after treatment as shown by the statistically significant effect of the contrast factor (Blue: $p<0.001$; Yellow: $p<0.001$; Red: $p<0.001$; Green: only two contrasts), but the interaction between group and contrast was not statistically significant for any of the colours.

Conclusions: The results showed that although hypothyroidism affects the temporal response to colour stimuli especially for low contrasts in blue and yellow regions, this process is reversible. This indicates that RT improved significantly after treatment for blue and yellow stimuli in particular and it became similar to controls. This might be due to the recovered function of the cells with S-cone input impaired by hypothyroidism possibly by a process of remyelination of neurons in the visual pathways.

FUNCTIONAL CONNECTIVITY IN DEVELOPMENTAL DYSLEXIA

Tihomir Taskov[1], Juliana Dushanova[1]

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Developmental dyslexia (DD) is one of the most common developmental learning disorders characterized by an inability to acquire typical reading skills, diagnosed around second grade. The difficulty acquiring

reading-related skills of DD children, such as word reading accuracy, fluency, and comprehension cannot be explained by intellectual, neurological, or motor, sensory impairments, or poor learning. Children with DD have a significant delay in reading ability and experience than typically developing peers and a negative impact on individual access to higher education.

Objective: This study aims to discuss the use of technology in the study of developmental dyslexia. Longitudinal study examines whether functional brain measures are related to reading ability and disability (dyslexia) in young children, how they develop over time, and in the context of a multiple deficit model that illustrates reading disability as the result of multiple risk and protective factors.

Methods: The most studied neurocognitive hypothesis for DD is those for the impairment of the ability to perceive and manipulate the sounds of spoken words. Other influential hypothesis concerns visual and/or auditory dysfunctions affecting reading acquisition. This study includes different perspectives on dyslexia from different contexts of the research, the results of which have the potential to improve the experiences of people with dyslexia.

Results: Understanding the early trajectories of reading development, behaviorally and in the brain, allows a better understanding of the etiological basis of reading disabilities and helps inform early screening, identification, and remediation, and is essential to enable the development of individualized neurocognitive stimulation, remediation and intervention strategies. The experiments in the study investigated learning-related changes in task-specific functional connectivity in normal readers and a group with DD, in which poor abilities in reading are related to several brain regions served cognitive processes, important to read. Specialized neural circuits in visual, auditory, sensory and motor processing, along with complex brain networks engaged in language and executive functions, coordinate the reading. Children with DD show functional differences in the left-lateralized complex brain network

that is remapped for reading acquisition. Intensive training induced changes in functional connectivity between and within neural networks important for literacy skills.

Conclusions: The study highlights the importance of taking a capability-based approach rather than a deficit-based one. Since crucial for finding employment and social acceptance are the reading abilities, the development of specific training to overcome these deficits is an important part of these studies. The study could attempt to translate core neuroscience research on brain and learning to the psychologist, logopedist, parents, and government policy.

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LONG COVID-19 SYNDROME IS ASSOCIATED WITH A PERSISTENT IMPAIRMENT OF SLEEP

Silvia Koumanova, Vasil Kolev, Lora Lyamova, Roumen Kirov, Juliana Yordanova

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: The COVID-19 pandemic has been shown to have a significant impact on sleep – amount of sleep, sleep quality, sleep patterns, and dreaming. Two major factors are accounted for such sleep problems: psychological and neurobiological. Psychological sources stem from the increased level of individual stress. The fear of becoming infected, the disease itself, mandatory lockdowns, and quarantines have led to major consequences inducing changed life styles, restricted social communication, depression, anxiety, and post-traumatic stress disorder, all linked to sleep impairments. On the other hand, in COVID patients, sleep impairments can be caused by neuroinflamation and damage in the central nervous system.

Notably, patients affected by COVID have shown impairments of sleep in both the acute and post-acute (long COVID) stage of the disease. Since the effects of COVID pandemic and disease on sleep have not been evaluated in Bulgaria so far, the following objectives were formulated in the present study. **Objectives:** (1) To provide original data on the effects of COVID and severity of long COVID on sleep parameters in a large Bulgarian sample; (2) To explore the recovery of sleep according to pre-pandemic levels, and (3) To assess the effects of infection vs pandemic stress on sleep in different COVID conditions. Here, the disease outcome was defined as short COVID, moderate, or severe long COVID as manifesting none, one (according to WHO definition) or at least three long-COVID-related symptoms over at least three consecutive months after the disease.

Methods: Data of the Bulgarian sample was collected in May-October 2021 from 737 respondents (405 valid cases) who participated in the second survey of the International COVID Sleep Study (ICOSS-2) through an internet-based platform. Sleep parameters (sleep duration, sleep quality, morning sleepiness, day sleepiness, day fatigue, and nightmares) were evaluated for four periods – before pandemic, during pandemic, during infection, and current state. ANOVAs and regression analyses were applied.

Results: In none of the groups, a full recovery of sleep quality was observed at the time of the survey. Sleep duration (insomnia) and day sleepiness remained compromised in all patients with COVID. Day fatigue, morning sleepiness and nightmares remained additionally impaired in severe long COVID patients who also manifested the lowest levels of recovery for each sleep parameter. Sleep quality was especially sensitive to pandemic stress in all groups. For all COVID conditions, deterioration of sleep was significantly increased during disease-related as compared to pandemic-related stress.

Conclusions: These results confirm that sleep is especially vulnerable to COVID pandemic and disease stress in a Bulgarian sample. Given the strong

association between mental and immune status and sleep, they also indicate that special care should be taken with respect to sleep problems in patients with long COVID after the disease, especially those with severe long COVID.
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PERFORMANCE MARKERS OF IMPLICIT LEARNING OF SENSORIMOTOR REGULARITIES

Violeta Valkova, Vasil Kolev, Juliana Yordanova

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Goal-directed behaviour critically depends on the level of consciousness and attention. However, an enormous part of the incoming information remains subconscious. This implicit knowledge, although remaining with little or no control on its implementation, may determine decision making, success of learning and creativity. Also, it is at the core of many psychiatric disorders like anxiety, phobias, etc. Implicit knowledge (ImK) of environmental regularities is of special relevance. If people acquire such knowledge, they can substantially improve their performance although they remain fully unaware of why this has happened. The gain of explicit knowledge (ExK) produces similar results but people are fully aware and can apply deliberate strategies to further improve their achievements. To explore how ImK is acquired, specific sensorimotor tasks with a hidden regularity are used. Interestingly, only some subjects can become aware of the hidden regularities, which drastically improves their performance. It is hypothesized that the accumulation of ImK is the major source of ExK generation. Therefore, understanding how ImK is accumulated in the course of learning and if it is generated in each individual is a most relevant scientific target. Due to methodological constraints, ImK has been only assessed at a group level which

does not permit to identify its role for bringing knowledge to awareness.

Objectives: To develop a methodological tool enabling the assessment of ImK accumulation at individual level.

Methods: A lateralized variant of the visual serial response time task (lSRTT) was used. The design included 3 parts composed of structured and random blocks. One of four different colours appeared on each trial and had to be responded to by pressing the appropriate key. Unknown to the participants, each part was a "sandwich" where the trials in the outer blocks were random, whereas the inner trials repeated a fixed sequence of 12 stimuli. The lSRTT was practiced in implicit conditions by 109 participants. ExK about the covert sequence was tested thereafter. The normalized difference between reaction times (RTs) in the last random block and the RT in the preceding ordered block was analyzed for each part. By using single trials, this difference was computed for each subject and tested using Student t-test. The presence of a significant increase was adopted as index for ImK generation at individual level and was termed ImK coefficient.

Results: Major results demonstrate that (1) All participants who became aware of the sequence (15.5%) gained ImK about regularities at learning, (2) ImK also was accumulated during learning by 40% of participants who remained fully unaware of regularities. (3) Sixty percent of subjects gained neither ExK nor ImK.

Conclusion: Developing and applying the ImK coefficient as a tool for individual quantification of ImK helped to reveal that less than half of the people are able to learn implicitly regularities. However, this ability supports the explicit awareness of such regularities thus promoting creativity and abstraction. Future research should definitely address the sources of these individual differences.

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ALTERATIONS OF MOTOR CORTEX CONNECTIVITY IN AGING

Juliana Yordanova, Konstantin Beshkov, Plamenka Nanova, Vasil Kolev

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: It has been demonstrated that during motor responses, the activation of the motor cortical regions is associated with the generation of strongly synchronized electroencephalographic (EEG) oscillations from theta (3.5-7 Hz) frequency range. Synchronized theta activity also is observed at medial frontal regions implicated with performance monitoring and cognitive control. There is evidence that motor cortical activation emerges in close association with the activation of the medial frontal cortex. A distributed oscillatory system in the brain operating in the theta frequency range has been suggested to play a major role in synchronizing cortical and sub-cortical brain regions during action monitoring. Theta network deficiencies may therefore be a source of dysregulated motor cortical functions.

Objectives: The major objective of the present study was to explore the effects of human aging on the neurodynamics and connectivity of motor EEG theta activity during correct motor response generation.

Methods: Response-related potentials (RRPs) were recorded from young and older adults while they performed sensorimotor tasks with different level of complexity of the stimulus-response association: a simple reaction task, a Go-Nogo task and a four-choice reaction task. Motor responses were given with the right hand. EEG was recorded at 64 electrodes. EEG signals were spatially enhanced using CSD. Trials from correct responses were analyzed. RRPs were decomposed in the time-frequency (TF) domain by means of wavelet transform. To assess connectivity, the phase-locking value (PLV) reflecting spatial synchronization between cortical regions was computed and analyzed in the theta frequency band for each pair of electrodes. The

PLV of all pairs guided by the motor cortex (C3 electrode) and FCz (medial frontal electrode) was measured and statistically analyzed to compare the connectivity of these two regions between young and older adults. ANOVA was employed for assessing aging-related differences.

Results: During motor response production there was a strong synchronization of theta oscillations between the mid-frontal-central region and contra-lateral sensorimotor regions demonstrating a functional connection between the medial frontal cortex and activated motor areas. Aging was associated with a significant reduction of PLV for all tasks. Motor response generation also was accompanied by an increased connectivity of the left motor regions contra-lateral to the response. In young adults, the activated motor cortex was most strongly connected with pre-motor and frontal regions of the left hemisphere. In contrast, motor cortex of older adults was connected with sensorimotor regions.

Conclusion: Increasing age in humans affects the connectivity between medial frontal and motor cortical regions during response generation suggesting aging-related alterations of the performance monitoring and adaptation network. Also, the altered connectivity of the activated motor cortex points to a dysregulation of motor cortical functions.

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EFFECTS OF VALORPHIN AND ITS NEWLY SYNTHESIZED AMINOPHOSPHONIC ANALOGUE ON CARRAGEENAN-INDUCED PAW OEDEMA AND HYPERALGESIA

Elena Dzhambazova[1], Borislav Assenov[1,2], Polina Mateeva[2], Teodor Slavchev[1], Paraskevas Pakataridis[1], Philippos-Savvas Chelmis[1], Nikol Hristova[1], Daniela Pechlivanova[1,2]

[1]*Medical Faculty, University of Sofia "St. Kliment Ohridski", Sofia, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*

Background: In the body, after eryptosis, the β-chain of hemoglobin produces short-chain peptides called hemorphins. They have opioid receptor affinity and morphinomimetic properties. One of these endogenous peptides is valorphine (V1). Literature data show that hemorphin peptides are increasingly used in the treatment of various diseases such as hypertension, epilepsy, diabetes, chronic pain, cancer, etc. An aminophosphonic analogue of valorphine (V2p) was synthesized in order to obtain a non-toxic substance with a more effective analgesic and anti-inflammatory effect.

Objective. The aim of the present study was to evaluate the effects of a local injection of V2p on the carrageenan-induced paw oedema and hyperalgesia.

Methods: V2p was injected intraplantar at a dose of 50 µg/ 5 µl/ rat before the inflammation. The inflammatory process was induced by the intraplantar injection of carrageenan lambda [CRG, 1% carrageenan in saline solution, 100 µl] into the right hind paw. The paw oedema was measured at the 1st, 3rd, and 4th hour after the carrageenan injection with a plethysmometer (Ugo Basile, Italy). Acute nociception is assessed using a paw pressure analgesimeter at the same intervals after the carrageenan injection. Indomethacin at a dose of 5 mg/ kg, injected intraperitoneally was used as a reference anti-inflammatory drug.

Results: The results showed that the CRG-induced inflammation decreased the mechanical pain threshold in the ipsilateral paw. V2p significantly reduced the hyperalgesia at the 1st and 3rd hours after the injection of CRG with an

antinociceptive activity comparable to indomethacin. The pretreatment with the valorphin analogue also significantly decreased CRG-induced paw oedema at the 1st and 3rd hours. The anti-inflammatory effect of V2p at 1st hour was similar to that of indomethacin, but less at 3rd and 4th hours after CRG.

Conclusions: The aminophosphonic valorphin analogue showed a well-expressed local antinociceptive and anti-inflammatory effects.

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ROLE OF THE PROTECTIVE RAS ARM IN THE REGULATION OF THE COGNITIVE BEHAVIOR AND MEMORY

Daniela Pechlivanova[1], Borislav Assenov[1,2], Paraskevas Pakataridis[2], Philippos-Savvas Chelmis[2], Elena Dzhambazova[2]

[1]Institute of Neurobiology, Bulgarian Academy of Sciences,Sofia, Bulgaria [2], Medical Faculty, University of Sofia"St. Kliment Ohridski", Sofia, Bulgaria

Background: The renin angiotensin system (RAS) is a functional structure that maintains homeostasis and brain functions through two distinct axes: ACE/Ang II/AT1 and ACE2/Ang-(1-7)/Mas R. Angiotensin II (Ang II) exerts its main effects by binding to the AT1 receptor. AT2 receptors together with the Mas receptor are functional antagonists of the AT1 receptor and a balancing arm in the RAS. Spontaneously hypertensive rats (SHR) are characterized by the development of hypertension, reduced anxiety, attention, nociception and increased activity compared to normotensive rats. Endogenous RAS imbalance is among the causes of these abnormalities in SHR.

Objective: The aim of the present study was to elucidate the effects of chronic activation of brain angiotensin AT2 receptors on anxiety and exploratory behavior, working and spatial memory in female SHR.

Methods: The objective was accomplished using the following tests: Open Field and Elevated Plus Maze to investigate exploratory and anxiety-like behavior, novel object recognition tests, and T-Maze for working and spatial memory. The selective peptide AT2 receptor agonist novokinin was infused intracerebroventricularly using osmotic minipumps and a stereotactically implanted brain set. All tests were performed twice, at the end of the infusion and three months later.

Results: Female SHRs demonstrated hyperactive and anxiolytic-like behavior, fewer correct alternations in the T-maze test which correlates with their impulsive behavior and no alterations in recognition memory. Novokinin-treated rats showed reduced anxiolysis and hyperactivity as well as improved habituation to the new environment, and spatial memory without changes in novel object recognition as compared to controls.

Conclusions: Female SHRs have behavioral and memory disturbances that involve, at least in part, a compensatory mechanism through the activation of brain AT2 receptors.

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VISCOELASTIC CHARACTERISTICS OF AORTIC IN VITRO PREPARATIONS FROM SHAM-OPERATED AND PINEALECTOMISED RATS

Andreyan Georgiev, Lyudmila Shikova, Maria Kaneva, Maria Antonova

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Background: Biomechanical behaviour of arteries has been studied extensively and various influencing factors have been described. One of these factors with great importance is the hypertension. Over time, higher arterial pressure and shear stress cause adaptive structural changes in the

arterial wall, which lead to adjustments in its viscoelasticity such as increased stiffness and decreased compliance. Knowledge of how far arteries change led to the adoption of arterial viscoelasticity as a predictor for diagnostics and prognosis of vascular disease. Melatonin is a neuroendocrine hormone that has effects not only in the central nervous system, but also throughout the rest of the body. Its peripheral effects are mediated by MT_1 and MT_2 receptors, which are also known to be expressed in cells of the cardiovascular system. Specifically, expression of MT_1 receptors has been confirmed in the outermost *tunica adventitia* layer of the rat aorta, both at mRNA and protein level. Studies have shown that melatonin improves cardiovascular functions, and it has also demonstrated anti-inflammatory and antioxidant properties. Melatonin decreases arterial blood pressure as well as heart rate; there is evidence that its secretion is inversely associated with arterial stiffness.

Objective: To estimate aortic viscoelasticity of *in vitro* preparations from sham-operated and pinealectomised rats and to compare their viscoelastic characteristics.

Methods: In this research, we evaluated two groups of male 4-month-old Wistar rats – one sham-operated (sham group) and the other pinealectomised (pin group). Each animal was sacrificed, and the obtained aorta immersed in a nutrient medium. The artery was then spirally cut into two strip preparations according to their respective anatomical position, i.e., thoracic and abdominal. Consecutively, each strip preparation was suspended in an organ bath chamber and its vitality was kept in quasi-physiological conditions during the experiment. Here, we used the forced oscillations' method for measurement of resonance curves and calculation of three viscoelastic characteristics – resonance frequency (f_0), dynamic modulus of elasticity (E') and coefficient of viscosity (β). Values were obtained at four pre-defined levels of arterial pressure in the range of 80-157 mmHg.

Results: E' and β were found to be significantly different between pin and

sham groups. The pin group showed lower E′ across all levels of arterial pressure. The sham group, on the other hand, had lower β at all pressure levels. Regardless of pineal status, mean values of E′ in the thoracic aorta were slightly lower than those in the abdominal section, while mean β were slightly higher.

Conclusions: Pinealectomy leads to increased distensibility and viscosity of the aorta, compared to physiological conditions. This finding is opposite to the expected increase of arterial stiffness in absence of melatonin, suggested in previous studies. Still, apart from melatonin, another mechanism of pineal influence on the rat aorta could not be ruled out.

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CARDIAC ARRHYTHMIAS IN PATIENTS WITH SLEEP APNEA SYNDROME

Darko Simonov[1,2], Anelia Dimitrova[1,3], Mario Milkov[1,4]

[1]Department of Physiology, Pathophysiology and Pharmacology, Faculty of Medicine, Trakia University - Stara Zagora, Bulgaria
[2]MHAT "Heart and Brain", Burgas, Bulgaria
[3]Department of Pathophysiology, Medical University of Pleven, Pleven, Bulgaria
[4]Faculty of Dental Medicine, Medical University of Varna, Varna, Bulgaria

Obstructive sleep apnea harms sleep and health in about 2-4% of the adult population. Proceeds with repeated respiratory breaks during sleep, alternating with strong snoring.

Increased number of wakes leads to incomplete sleep with excessive daytime sleepiness, decreased working capacity, memory disorders, attention, concentration. Stopping breathing puts the body in conditions of hypoxia and increased stress. Hipoxia demages all organs and systems- first of all the

cardiovascular and endocrine.

In the period 01/2017 – 06/2022 our team has diagnosed obstructive sleep apnea syndrome in 104 patients using polygraph testing at home. Patients were followed by clinical examination, measurement of arterial pressure, electrocardiogram, echocardiography, BMI, in order to assess the presence of cardiovascular complications (arterial hypertension, arrhythmias, ischemic heart disease, heart failure).

The frequency of cardiac arrhythmias, mainly nocturnal, increases with the increased severity of sleep apnea/hyponea syndrome. The most common heart rhythm disorders observed in patients with sleep apnea/hyponea syndrome are sinus bradycardia, sinus pause, first-degree AV block, second degree type Mobitz I, increased number of PVCs and atrial fibrillation.

STUDY OF BODY COMPOSITION AND OBESITY IN CHILDREN AGED 12-14 YEARS

O. Hristov; M. Zdravcheva; P. Peev; P. Petkova

National Sports Academy "Vasil Levski", Sofia, Bulgaria

Background: In the last two years, the topic of motor activity and the consequences of lack of movement has been particularly relevant. According to data from the Metropolitan Health Inspectorate, the health status of students in grades 7-12 shows that overweight and obesity are a major problem for most age groups. We assume that the main risk factors are irrational nutrition and low physical activity. On the other hand, the presence of overweight, obesity and reduced physical activity among children and students are risk factors for the occurrence of chronic non-communicable diseases at a later age.

Objective: The aim of this study is to determine the body composition and obesity of the examined children and relate it to their physical activity. The

study group included 317 girls and boys with a mean age of 12.8 years ±0.8 months.

Methods of the research are body composition analysis and statistical procedures. The body composition of the subjects was determined using the InBody 230 system, which has 8-point tactile electrode system with direct segmental analysis, which measures impedance for the five different parts of the body at multiple frequencies. Descriptive statistics of the results included mean value and standard deviation.

Results: The results of the study showed that 10.4% of all measured children were overweight (boys = 11.2%; girls = 9.8%). Also, 93.9% (boys = 42.4%; girls = 51.5%) have a percentage of body fat above the norm and 54.5% (boys = 45.9%; girls = 9.1%) have a degree of obesity above the norm. Normal body mass values were observed in 53.3% (boys = 21.1%; girls = 32.2%) of the subjects, but in 34.9% (boys = 17.8%; girls = 17.2 %) has elevated values of body fat percentage and in 19.5% (boy=7.7%; girls=11.8%) the degree of obesity is above the norm.

Conclusions: In conclusion, it can be said that only 17.7% of children have a degree of obesity above the normal ones. 19.5% of children with normal body weight are obese, which would suggest an unhealthy diet and low duration of physical activity.

ADIPOKINES AS BIOMARKERS FOR DIABETIC COMPLICATIONS

T. Apostolova, Petya Hadzhibozheva, Tsvetelin Georgiev

Department of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

The white adipose tissue - one of the largest organs in the body, nowadays is viewed as a complex structure, not only responsible for energy storage

and lipid metabolism, but also producing hundreds of biologically active substances, generally named adipokines. They play a significant role in many physiological processes such as appetite regulation, glucose metabolism, immune function and cardiovascular homeostasis. The increased adipose tissue mass in obesity is associated with adipokine levels imbalance. Furthermore, some adipokines, through their effects on insulin resistance, vascular function and inflammation, have been linked to the pathogenesis of obesity and obesity-related diseases such as type 2 diabetes mellitus (T2D). Among them, the newly discovered visfatin and asprosin have been found to be at higher levels in diabetic patients, suggesting that they may have a pathogenic role. Conversely, adiponectin could serve as a protective factor.
This study summarizes the knowledge about the role of these adipokines as factors in the development of T2D as well as their ability to serve as biomarkers for diagnosis of diabetes and the prognosis of the severity of its complications.

PHYSIOLOGICAL ASPECTS OF BURNOUT SYNDROME

Viktoriya Dyakova, Petya Hadzhibozheva, Tsvetelin Georgiev

Department of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

Burnout is a syndrome resulting from the lack of recovery from long-term stress at work. It possesses the following three basic characteristics: 1) emotional exhaustion; 2) depersonalization (cynicism); 3) low personal accomplishment. The negative impact that burnout has on public health has led the World Health Organization to include this syndrome in the most recent International Classification of Diseases (ICD-11), which considers burnout as a phenomenon in the occupational context. Burnout syndrome is prevalent in modern societies, and the fact that it takes time to develop,

makes intervention possible at every stage. Studies show that the symptoms of burnout can be both psychological and somatic.

Our presented work summarizes some findings on the physiological aspects of burnout, as they are manifested through physical symptoms and changes in biological parameters. In patients with clinical burnout one of the main characteristics is dysregulation of the hypothalamus-pituitary-adrenal axis and dysfunction of the autonomic nervous system. Thus, physical consequences of burnout syndrome might include potentially life-threatening conditions, such as cardiovascular disease, immunosuppression, chronic inflammation, metabolic syndrome and functional brain changes.

Therefore, a need arises for the establishment of clear biological markers for early diagnosis of burnout syndrome and its differentiation from depressive disorders. If more research is directed towards analyzing the physiological and pathophysiological aspects of the syndrome, then it would be possible to diagnose it properly and prevent its further development.

STUDY ON THE RELATIONSHIP BETWEEN THE CONTENT OF VITAMIN D IN THE BODYAND BONE DENSITY

Hristina Shtereva, Pavlina Teneva, Ivelina Dobreva, Katya Kichukova, Nafize Bekir, Steliana Valeva, Anna Tolekova

Medical college, Trakia University - Stara Zagora

Background: Two main components underlie bone strength – bone tissue and bone density. In this connection, vitamin D plays an important role. Its deficiency is directly related to osteoporosis. Such deficiency is very likely to lead to osteopenia, osteoporosis and osteomalacia. All of these conditions are associated with muscle weakness that leads to falls and fractures. Osteopenia is a condition of reduced bone mineral density. Osteoporosis, on the other

hand, is characterized by deteriorated bone microarchitecture, which also leads to bone fragility and a serious risk of fractures. The thinning of the bone is the result of the lack of balance between bone formation and degradation, as the degradation takes precedence and the bone becomes more fragile. In this sense, vitamin D has an important role in these processes and can lead to the prevention or treatment of osteoporosis.

Objective: Purpose of the study - correlational interactions between bone density and concentration of vitamin D, some hormones and some biochemical constants.

Methods: Healthy individuals aged from 19 to 45 years were studied. They are divided into two groups depending on the bone density – normal and with osteopenia. Vitamin D, Angiotensin II, Osteocalcin and liver enzymes (ASAT, ALAT, GGT, ALP) were studied.

Results: They are established positive correlation with significant differences between osteodensitometry results and ALAT, ASAT, GGT, ALP, Osteocalcin (ng/ml). They are no significant differences in the various of vitamin D of the two groups.

Conclusions: The analysis of osteocalcin data shows that remodeling processes in the direction of bone loss are activated in the group with osteopenia.

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