2025

BOOK OF ABSTRACTS

NATIONAL SCIENTIFIC CONFERENCE

NOVEMBER 24-25
Sofia

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Contents

Scientific session 1:

NEUROANATOMY, NEUROPHARMACOLOGY

MYENTERIC PLEXUS MORPHOLOGICAL DIFFERENCES IN THE RIGHT AND LEFT COLON AND ITS POSSIBLE CLINICAL SIGNIFICANCE

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Colorectal cancer (CRC) is the fourth most common malignancy worldwide and shows a rising incidence in young adults. Its prognosis is strongly influenced by tumor location as well as by interactions with the enteric nervous system, particularly neurotransmitters and neuropeptides. This study aimed to provide a detailed morphological characterisation of the myenteric plexus in the right and left colon (RC, LC) of 3-month-old Wistar rats and to relate these features to the poorer prognosis typically associated with right-sided neoplasms. We found that the mean neuronal perikaryal area in the RC was 442.3 μm², approximately 25% larger than in the LC. Ganglia in the RC were also significantly larger (approximately 40% larger). Neuronal density showed a pronounced difference, with 153.9 ± 49.48 neurons/mm² in the RC compared with 58.13 ± 16.81 neurons/mm² in the LC. Furthermore, collagen fibers encapsulating the myenteric ganglia were nearly 40% less abundant in the RC than in the LC. These marked structural differences in the myenteric plexus between the right and left part of *intestinum crassum* may, at least in part, underlie the association between tumor location and CRC prognosis.

Key words: Enteric nervous system (ENS), myenteric plexus, neuronal morphology, right- and left-sided colon, collagen fibers, colorectal cancer (CRC)

NOVELTY-INDUCED ENHANCEMENT OF INITIAL CONSOLIDATION OF OBJECT AND CONTEXTUAL THREAT MEMORIES IN THE ANTERIOR RETROSPLENIAL CORTEX

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The behavioral tagging hypothesis (BT) has provided a robust theoretical framework for studying the modulatory effects of novelty on the neurobiological mechanisms of initial consolidation of memories for emotionally neutral or low-arousing events. Specifically, studies supporting BT have demonstrated the existence of novelty-induced retroactive and proactive enhancement on initial memory consolidation in the dorsal hippocampus (dHPC), which has been associated with increased release of the catecholaminergic neuromodulators dopamine and noradrenaline. It is believed that these signaling molecules trigger the synthesis of plasticity-related proteins that are required for the long-term stabilization of memory traces. Considering the presumptions outlined by the BT hypothesis, our research group set out to examine possible enhancing effects of novelty in another brain structure with a pivotal role in episodic-like memory formation in rodents - the anterior retrosplenial cortex (aRSC). Much like the dHPC, aRSC is active during memory consolidation and early retrieval of memories for events taking place in the environment. To obtain more extensive view of plausible novelty-induced enhancement of initial consolidation in the aRSC, we performed two separate experiments that took advantage of two different behavioral protocols designed to evaluate memory in rodents - the spontaneous object recognition task and a low-intensity version of the contextual threat conditioning. We integrated these protocols with a short exposure to environmental enrichment (EE) cage scheduled 20 to 30 minutes upon initial behavioral training. The EE exposure was used as modulatory events capable of increasing dopamine levels in aRSC due to its high degree of novelty. Additionally, after the completion of the EE session in both experiments, two groups of animals were subjected to direct infusion of the dopamine D1 receptors antagonist, SCH 23390, or the protein synthesis inhibitor, Anisomycin, in the aRSC. The test phase of the behavioral tasks was performed 24 hours after the pharmacological manipulations. During test phase, we observed substantially better performance in animals that were exposed to novelty and were not subjected to pharmacological manipulations. Overall, our experiments provide the first evidence for novelty-induced enhancement of memory consolidation in aRSC, similar to what was previously reported in dHPC.

Keywords: memory consolidation, novelty, environmental enrichment, anterior retrosplenial cortex, dopamine

Acknowledgement: This work is funded by the National Science Fund of Bulgaria (research grants: КП-Н06-81/7; КП-06-Н61/12)

PHARMACOLOGICAL STUDIES ON NEWLY SYNTHETISED MAO A AND B INHIBITORS ON MPTP MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the selective loss of dopaminergic neurons in the substantia negra, leading to dopamine deficiency in the striatum and consequent motor impairments. Beyond motor symptoms, the disease involves widespread neurochemical alterations and inflammation.

The aim of our study was to investigate the efficacy of three newly synthesized indole-based hydrazide hydrazone and derivatives, **3e, 3f** and **3i** on motor deficiency and neuroinflammation induced by MPTP in a mouse model of PD. The three compounds were ip injected (10 and 50 mg/kg, 5 days), and treatment with MPTP /20 mg/kg, 4x every 2 hours/ started on the 3rd day. Neurological tests were used to investigate their protective activity, and biochemical methods to determine their effect on neuroinflammation markers in striatum and hippocampus.

Treatment with **3e, 3f,** and **3i** mitigated the motor dysfunction and muscle strength caused by MPTP as measured by the rotarod and the actimeter. Positive effects on MPTP-induced neurological abnormalities correlated with reduced expression of tumor necrosis factor-alpha /TNF-α/, interleukin-beta /IL-β/ in the hippocampus and dopamine levels in the striatum. Similar effects were found for the reference substance, selegiline.

Our results suggest that the three novel hybrid compounds characterized by dual inhibition of MAO-B and MAO-A are a promising approach to ameliorate PD symptomatology through neuroprotection and anti-inflammatory activity.

Keywords: Parkinson's disease; dopamine; indole-based hydrazide hydrazone derivatives; neuroinflammation; striatum

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STRUCTURE-DEPENDENT EFFECTS OF NEWLY SYNTHESIZED ANG 1-7 PEPTIDE ANALOGS IN AN IN VIVO MODEL OF CHEMICALLY INDUCED NOCICEPTION IN MICE

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The heptapeptide angiotensin 1-7 (ANG 1-7) is a key member of the ACE2/ANG 1-7/Mas1R axis, or so-called balancing arm of renin–angiotensin system (RAS), opposing the effects of the classic axis (ACE/AngII/ AT1R) mediating its homeostatic and neuromodulator functions. On the other hand, classic axis involves octapeptide Angiotensin II mediating vasoconstriction, proliferative, and pro-inflammatory effects via binding to AT1-type receptors throughout the body. Moreover, it is involved in nociceptive behavior. Ang 1-7 effects oppose the effects of Ang II via MAS1 receptors and more specifically inhibit p38 MAPK activation and subsequent pain-related behaviors. Based on scientific literature data, our study aimed to investigate mechanisms of antinociception induced by Ang1-7 and its four structural synthetic analogs in the formalin test in mice. For the purposes of the study, four new ANG 1-7 synthetic analogs - A1, A2, A3, and A4 were synthesized and characterized by electrochemical, spectral, and other methods. New synthetic derivatives were modified with unnatural amino acid modifications on the N-terminal.

Male ICR mice were distributed in three groups administered intraperitoneally (i.p.) with Ang1-7 synthetic peptides: 1/ at a dose of 1mg/kg; 2/ at a dose of 0.5 mg/kg; and 3/ at a dose of 0.25 mg/kg. Formalin test was used to evaluate the acute phase and inflammatory phase of nociception. The stereotype responses of licking or shaking the injected paw were observed and measured in seconds, separately for each phase of the experiment.

Results from the acute nociceptive phase showed that at a dose of 1 mg/kg peptides A1, A3, and A4 did not demonstrate antinociception, and A2 showed a significant antinociceptive effect. At a dose of 0.5 mg/kg, peptides A1, A4, and A3 had no effect, but A2 produced a pronounced antinociceptive effect. In the third group, we didn't observe any antinociception activity compared to the control group. Results from the inflammatory phase in the first experimental group showed that peptides A1, A3, and A4 did not demonstrate antinociceptive effects. The only exception was peptide A2, which showed significant antinociceptive activity. At a dose of 0.5 mg/kg, A3, A2 and A4 had no reduce the nociception, and A1 produced an antinociceptive effect with statistical significance. In the third group, we observed the same effects of the tested peptides.

Conclusions - Present data showed a well-defined antinociceptive effect of peptide A2 at doses of 1mg/kg and 0.5mg/kg during the acute nociceptive phase of the formalin test, as well as in the inflammatory phase at a dose of 1mg/kg. The same conclusion is valid for peptide A1, which demonstrated an antinociceptive effect in the inflammatory phase at doses of 0.5mg/kg and 0.25mg/kg. The last two synthetic peptides - A3 and A4 didn't demonstrate any antinociceptive activity, neither in the first phase, nor in the second phase. This complex result could be explained by further and deeper analysis of the relation between the structural features of the synthetic Ang 1-7 analogs and the pharmacological effects they produce.

Acknowledgments: This work was financially supported by the Bulgarian National Scientific Fund project KP-06-H71/9 of the Ministry of Education and Science, Bulgaria.

Scientific session 2:

NEUROPHYSIOLOGY

EVENT-RELATED POTENTIALS REVEAL DIFFERENTIAL MODULATIONS OF IMPLICT PROBABILITY CONTEXT ON PROACTIVE AND REACTIVE MOTOR CONTROL

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Background: Implicit contextual influences on motor processing are mediated by proactive and reactive control. The proactive control is implicated in predicting and planning responses in order to prevent interference and optimize behavior. It operates by inducing preparatory cortical states before the relevant movement. The reactive motor control supports response initiation and interference resolution after movement onset. It is substantiated by processes of continuous action monitoring and cognitive assessment which implement ongoing adjustments and corrections. Given this complex interplay between proactive and reactive control, the associations between movement expectation/preparation, execution, monitoring and cognitive evaluation have gained increasing interest.
Objective: The aim of the present study was to assess the implicit effects of motor target probability on pro-active motor preparation as reflected by negative slow potentials (NSPs), motor execution as reflected by response-related potentials (RRPs), performance monitoring as reflected by correct response negativity (Nc), and cognitive motor target evaluation as reflected by P3b component of event-related potentials (ERPs). It was hypothesized that if ongoing increases/decreases in preparation induced by target expectation modulate motor execution, monitoring, and evaluation, there would be an association in the effects of motor target probability on NSPs, RRPs, Nc, and P3b.
Methods: Electroencephalographic (EEG) signals were recorded at 32 electrodes in 27 young adults while they produced simultaneous responses with the two hands to Go trials in auditory Go/NoGo tasks with different target probabilities (P = 0.15, P = 0.50, and P = 0.85). Reaction times were recorded for each hand. NSPs and RRPs were analyzed at the midline and bilateral motor regions, Nc was analyzed at medial frontal electrodes, and P3b was analyzed at parietal electrodes.
Results: Midline NSP increased with target probability increase pointing to enhanced preparatory attention with higher probability. The effects of probability on bi-lateral NSPs were lateralized: At the left motor cortex NSPs were expressed for the low and high probabilities, mirroring response speeding, whereas at the right motor region NSPs were not pronounced for higher (P = 0.50 and P = 0.85) probabilities. Bi-lateral RRPs were larger for low- than equal- and high-probability targets and correlated positively with left-hemisphere NSPs but negatively with right-hemisphere NSPs. Nc did not depend on target probability, whereas P3b increased with probability decrease.

Conclusions: Opposite dependencies of midline SNP and P3b on probability provide evidence for the inverse association between proactive and reactive control: weak proactive preparation is accompanied by intense reactive cognitive processing. The lateral and functional asymmetry of bi-lateral NSPs/RRPs suggest that the interaction between movement preparation and execution is modulated by hemispheric specialization in motor control, with the left hemisphere promoting execution in enhanced inhibition context, and the right hemisphere potentiating proactive inhibition in response generation context. Implicit motor response probabilities do not affect performance monitoring in Go/NoGo tasks as captured by time-domain Nc.

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DISTINCT PATTERNS OF DIRECTED CONNECTIVITY IN CONTENPLATIVE BRAIN STATES: A GRANGER CAUSALITY EEG STUDY

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Background. Contemporary neuroscience increasingly recognizes that contemplative brain states as represented by meditation do not modulate isolated brain regions in a uniform manner but rather orchestrate dynamic interactions within and between large-scale brain networks. Meditation practices engage large-scale networks that underlie self-referential processing, attentional control, and emotion regulation (Shen et al., 2020). These networks communicate through frequency-specific oscillations from different frequency ranges (theta, alpha, beta). Since specific meditative practices emphasize distinct cognitive and affective processes such as sustained attention, meta-awareness, emotional regulation, and prosocial affect (Cahn and Polich, 2006; Lutz et a., 2008) these processes may engage dissociable patterns of directed connectivity that can be analyzed by employing Granger causality (GC).
Objective. The present study applied spectral GC analysis to electroencephalographic (EEG) recordings obtained during Focused Attention Meditation (FAM - active focusing attention on a chosen object while inhibiting distractions), Open Monitoring Meditation (OMM - non-reactive awareness of moment-to-moment experience without focusing on a particular object), and Loving Kindness Meditation (LKM – maintenance of prosocial positive emotional states like compassion and empathy) in highly experienced meditators. The aim was to complement previous undirected connectivity observations (Yordanova et al., 2020) and explore if distinct directed connectivity signatures are associated with each meditation style. Such differences

were expected to highlight the neural grounds of the cognitive and affective state of each meditative practice.
Methods. Multivariate Granger causality was computed from high-resolution EEG signals recorded from long-term meditators (n = 22) in four conditions: rest, FAM, OMM, and LKM. GC was analyzed in the frequency domain with respect to the strength, frequency band, and direction of inter-regional information transfers for key cortical regions (anterior-left, anterior-right, posterior-left, posterior-right) to compare frequency-specific GC between rest and each meditation style.
Results. Each meditation state produced highly specific alterations in information transfer relative to rest. In FAM, there was a significant reduction in posterior-to-anterior GC in the alpha and beta bands, and decreased multi-spectral inter-hemispheric anterior GC pointing to attenuated bottom-up sensory and associative inputs. In OMM, multi-spectral GC was significantly increased from the left hemisphere to the right posterior cortex implying expanded awareness in the right posterior regions through enhanced top-down modulation by the left-hemisphere. The distinctive features of LKM profile were the inter-hemispheric symmetry, the posterior-anterior bi-directionality, and the specific beta-band engagement, implying a co-activation of systems that support an emotionally balanced stance, equanimity and pro-social attitude.
Conclusion. These novel findings demonstrate that the direction and frequency specificity of information flows provide complementary insights into neural processes underlying distinct meditative states.
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THE GAIN OF IMPLICIT KNOWLEDGE IS MODULATED BY NEUROCOGNITVE NETWORKS

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Background: When exposed to structured sensorimotor regularities, not all, but only some people can acquire implicit knowledge (ImK) about the abstract regularity. They improve their performance without conscious access to the learned material. It has been suggested that the formation of implicit memory requires practice-dependent strengthening of encoded structured engrams. We hypothesize that specific activation regimes of neurocognitive networks may additionally influence or interact with memory representations. Specifically, we hypothesize a role for the neurocognitive network for cognitive control and performance monitoring, which continuously compares ongoing behavior with the neural model of task goal. It operates in the theta frequency range through a fronto-medial (FM) hub. In addition, fronto-parietal (FP) attentional networks operating in the theta range may affect ImK accumulation. A dorsal attention system (bilateral) maintains focused, goal-directed attention.

A ventral attention system (right-lateralized) redirects attention toward novel, salient, or biologically relevant stimuli, with the right frontal cortex suggested to support the processing of abstract regularities.
Objective: The aim of the present study was to analyze functional network connectivity in order to test two hypotheses: (1) ImK is determined by the stabilization of sensorimotor memory engrams, and (2) ImK is determined by specific activation patterns of neurocognitive systems.
Methods: The lateralized visual serial response time task (SRTT) containing regular and random blocks was practiced in implicit conditions by 109 participants. Two groups of subjects were identified – who acquired implicit knowledge (ImK) and who did not (NoK). EEG was recorded from 32 electrodes. Phase-locking value (PLV) was computed to reflect functional connectivity: (1) Visual-motor PLV in theta, alpha, and beta bands to capture sensorimotor connections, (2) FM PLV in the theta band, and (3) FP PLV in the theta band to evaluate neurocognitive networks. NoK and ImK groups were compared in the beginning (T1) and in the end (T3) of SRTT learning.
Results: (1) No difference was observed between NoK and ImK for any frequency band in either T1 or T3. This result does not support the hypothesis that implicit knowledge arises from the strengthening of direct connections between sensory and motor cortices. (2) FM PLV: In T1, theta PLV was larger in ImK than NoK at parieto-occipital areas contra-lateral to the side of training suggesting that strengthened monitoring of visual information may serve as a precursor to the formation of implicit knowledge. In T3, theta PLV was increased at bilateral frontal areas only in ImK, reflecting facilitated communication between cognitive control and monitoring systems. (3) FP PLV: FP theta PLV was increased in ImK already in T1 suggesting that increased focused attention may serve as a precursor for the formation of implicit knowledge. In T3, there was a right-lateralized enhancement only in the ImK group, implying that the implicit model engages the ventral attention system, possibly in relation to attentional reorientation toward novel task features, such as a hidden regularity.
Conclusions: These results reveal that the mode of neurocognitive systems engagement plays a major role for the formation and maintenance of implicit representations of sensorimotor regularities.
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Scientific session 3:

NEUROPHYSIOLOGY

STATISTICAL LEARNING DURING ACTIVE ENGAGEMENT OF THE PARTICIPANTS' ATTENTION

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Statistical learning usually refers to an observational type of learning without task or feedback, which automatically and implicitly develops an internal structural representation of the spatial or temporal regularities in the sensory input (Fiser, Lengyel, Annu Rev Vis Sci. 2022, 15;8:265-290). The present study aimed to examine the role of attention in statistical learning. To achieve this, we selected a specific task that was not directly related to spatial regularities in the stimuli but required active engagement of participants' attention.
In our experiment, 16 out of 20 images were presented in various combinations 100 times on a monitor, arranged in a 4 x 4 grid. Two identical images appeared in the grid in half of the trials. Eight of the images were shown at the same position in the grid with a high probability (70-100%), whereas the positions of their counterparts in presentations with identical images were random. Each stimulus was displayed for 5000 ms. The participants' task was to indicate whether the stimulus contained a pair of identical images. In a follow-up experiment, the participants had to arrange 16 of the previously shown images in an empty grid using a computer mouse, at the positions where they appeared most frequently. Twenty-seven participants (21-70 years old; mean age 43 years) took part in the study.
The results showed that participants detected the identical stimuli with high accuracy, achieving an average percentage of correct responses of 80.1%. The median response time for presentations with identical images was 6094 ms, showing a tendency to decrease in successive experimental blocks, while it was 6239.5 ms for trials with unique images.
The participants reported difficulty in performing the follow-up experiment as they could not recall or arrange the images in the order they appeared most frequently. Nevertheless, the results showed that the participants managed to reproduce the positions of at least some of the images with high accuracy, even while engaged in a different task that required their attention and was unrelated to memorizing the images' positions. However, most participants were unable to reproduce the positions of two images (a circle and a square) occupying the same location in all trials. This issue might be due to the scanning strategy, either row-by-row or column-by-column. Since these images were presented in the middle of the grid, the participants may not have had enough time to look at the central part. Another possibility is that the circle and the square are the two most "impersonal" images, too familiar and somewhat similar to each other, and less semantically engaging compared to the other images. Further experiments are necessary to confirm the effects of position and semantic content. Also, the analysis of participants' eye movements could shed light on their strategy for performing the task.

INTERFERENCE OF THE SPATIAL CHARACTERISTICS ON THE CLASSIFICATION OF VISUAL STIMULI

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The ability to classify objects or events is a crucial cognitive process that facilitates a deeper understanding of the world by grouping similar items, thereby simplifying the complexity of incoming information. In the present study, we examined the ability to classify images with varying degrees of redness and clustering in categories based on either of these features or on their combination. This type of study requires forming a decision boundary between different stimuli based on their similarity and adjusting the decision rule in response to the provided feedback.

The study consisted of three experiments differing only in the classification rule. Participants were presented with black squares containing 24 red and yellow squares arranged in different groupings and color ratios. For each color ratio, we used 14 groupings, and for each grouping, we used all 14 color ratios, forming a set of 196 unique stimuli. Each stimulus was presented for 2 seconds, and during each trial, participants received feedback via a sound signal after providing their response.

In the first experiment, the stimuli had to be classified into two categories according to color dominance, regardless of the groupings. In the second experiment, classification was based on the number of groupings, regardless of color dominance. In the third experiment, classification was based on a combination of color dominance and the number of groupings. One category included stimuli that had either a greater number of groupings along with more yellow squares, or fewer groupings paired with fewer yellow squares. The other category consisted of stimuli that featured either more groupings with fewer yellow squares or fewer groupings with more yellow squares. Twenty-eight participants (aged 21–55) completed the experiments in a counterbalanced order, with at least one week between sessions.

Performance was assessed through accuracy and response time across seven blocks of trials. Results showed generally low accuracy, longer response times, and notable individual differences. Classification based on a single feature (color or grouping) was more accurate than classification using the combined rule. Accuracy improved when the number of clusters was high, especially for categories where redness and grouping changed in parallel. Stimuli with greater spatial dispersion were classified most effectively.

The results suggest difficulty in adaptively adjusting the decision boundary in multidimensional stimuli when the classification rule changes. They also imply that forming a decision boundary based on colorfulness is significantly influenced by the compactness of image elements and that the color and spatial characteristics of the stimuli interact in evaluating image similarity.

RELATIONSHIP BETWEEN PSYCHO-EMOTIONAL BALANCE AND POSTURAL CONTROL

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Recently is increasingly often stated that the human body and the psyche function as an integrated system in which the emotions and the postural stability interact two-way. It is known that the psycho-emotional balance shows the ability of an individual to regulate his emotions and to maintain the internal stability in stressful situations. Physiologically, this is related to the limbic system, the prefrontal cortex and the autonomic nervous system. On the other hand, to maintain the body in a stable, upright position, the integrative role of the CNS in relation to the afferent information coming from the three sensory systems – visual, vestibular and proprioceptive is of essential importance.

Functional imaging studies (fMRI, PET) confirm the two-way interaction between the vestibular stimulation and the activation of the amygdala, the insular cortex and the cerebellum, which leads to an intense emotional reaction of fear and anxiety. The amygdala, through direct neural projections to the vestibular center in the brainstem, affects the vestibular system and creates conditions for emotional states that influence the postural stability through changes in the muscle tone and the autonomic activity.

It is provided a general overview of the concepts that consider equilibrium as the ability to achieve simultaneous internal and external balance, also the main mechanisms through which the psycho-emotional processes influence the postural control, and analyses of the application of this knowledge in the clinical and practical activities. The analyses show that, although indirectly, the improvement or the deterioration of the cognitive functions and the physical state influence the postural control.

The aim of the literature review is to provide a theoretical basis for building a scientific hypothesis as to whether by improving the cognitive functions and increasing the physical activity we could significantly affect the psycho-emotional state and the postural control.

Keywords: *psycho-emotional balance, postural control, equilibrium, stability, internal and external balance*

Scientific session 4:

NEUROANATOMY, NEUROPHARMACOLOGY

STEREOLOGICAL ANALYSIS OF MICROVASCULATURE IN THE CATORID BODY OF SPONTANEOUSLY HYPERTENSIVE RATS

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The carotid body (CB) is the principal peripheral chemoreceptor responsible for monitoring arterial oxygen, carbon dioxide, and pH, thereby exerting a critical influence on autonomic cardiovascular regulation. Owing to its exceptional metabolic demands, the CB ranks among the most densely vascularized tissues, with vasculature constituting roughly one-quarter of its volume. While hypertension is known to induce structural and functional plasticity within the CB, quantitative characterization of its microvascular architecture remains incomplete. To address this gap, we performed an unbiased stereological evaluation of CB microvasculature in spontaneously hypertensive rats (SHR) compared with normotensive Wistar rats (NWR). High-resolution scanning electron microscopy enabled detailed reconstruction of the CB vascular bed, and stereological techniques were used to quantify total capillary length and mean capillary cross-sectional area. SHR demonstrated striking microvascular expansion, with total capillary length reaching 10.7 m ± 0.189, i.e. nearly double that of NWR (5.4 m ± 0.160). In contrast, the globally cross-sectional capillary caliber was markedly reduced in SHR (21 µm² ± 0.577) versus NWR (57.8 µm² ± 0.447), indicating a tightly packed, high-density vascular network characteristic of hypertensive remodeling. These data provide compelling evidence of angiogenic reorganization in the hypertensive CB, typified by elongation and redistribution of capillaries. Such structural adaptations likely augment chemosensory gain and may contribute to persistent sympathetic overactivity in hypertension. Our findings underscore the CB vasculature as a mechanistic nexus in hypertensive pathophysiology and highlight its therapeutic potential as a novel modulatory target for attenuating sympathetic drive in cardiovascular disease.

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ORAL EXPOSURE TO POLYSTYRENE MICROPLASTICS AND ITS IMPACT ON MALE REPRODUCTIVE FUNCTION IN RATS – SPERM MOTILITY AND KINEMATIC PARAMETERS

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Microplastics (MPs) represent an emerging class of persistent pollutants with proven cytotoxic and endocrine-modulating potential that have, over the past decade, become a significant factor of anthropogenic contamination. Their microscopic size, large specific surface area, and chemical stability render them highly bioavailable and prone to bioaccumulation within tissues. Among different polymers, polystyrene (PS) is of particular concern due to its widespread use, chemical resilience, and high bioaccumulative tendency, which facilitate its interactions with cellular membranes, enzymatic systems, and hormonal regulators. Prolonged exposure to polystyrene microplastics (PS-MPs) has been associated with oxidative stress, mitochondrial dysfunction, and endocrine disruption, leading to reproductive impairments. The aim of the present study was to evaluate the effects of a 52-day oral exposure to PS-MPs of different particle sizes (1 and 5 μm) at a dose of 0.1 mg/24 h on the functional and kinematic parameters of sperm in sexually mature male Wistar rats (2 months old). Spermatozoa isolated from the seminiferous tubules of the epididymis were analyzed using computer-assisted sperm analysis (CASA). Total and progressive motility (TM%, PM%) were assessed, together with velocity (VCL, VSL, VAP) and linearity parameters (LIN, STR, WOB). The results demonstrated that chronic exposure to PS-MPs led to a significant reduction in sperm motility and alterations in velocity parameters. A clear size-dependent effect was observed, with 1 μm particles causing more pronounced disruption of kinematic parameters compared to 5 μm particles. In conclusion, 52-day exposure to PS-MPs induced substantial impairments in sperm motility in Wistar rats, likely resulting from redox imbalance and endocrine dysregulation. These findings support the hypothesis that MPs act as potential reproductive toxicants and highlight the need for further research on their mechanisms of action, dose dependence, and reversibility of the observed effects.

Keywords: polystyrene microplastics, sperm motility, CASA, *Wistar* rats, reproductive toxicity

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EFFECT OF SHORT- TERM POLYSTYRENE MICROPLASTIC EXPOSURE ON ACETYLCHOLINESTERASE ACTIVITY IN MICE ORGANS

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Polystyrene is one of the most widely used plastics, with applications across various industries, including packaging, textiles, construction, and medical device manufacturing. Degradation of polystyrene and other polymers leads to the formation of micro- and nanoparticles capable of penetrating biological systems and accumulating in living organisms. Polystyrene microplastics (PS-MPs) have been detected in various organs and tissues of both animals and humans, where they can disrupt cellular homeostasis and induce structural and functional damage. Acetylcholinesterase (AChE) plays a key role in neural transmission and signaling, while in non-neuronal tissues it contributes to the regulation of diverse physiological processes, including maintenance of cell membrane stability, cell signaling, immune regulation, and responses to environmental stressors. The aim of the present study was to investigate the effect of short-term exposure to PS-MPs on acetylcholinesterase activity in mouse organs. Male and female SWISS albino mice were divided into two groups: Control and PS-MPs-exposed. The control group received purified water, whereas the exposed group received 1 μm PS-MPs suspended in purified water at a dose of 0.1 mg/day for 14 days. At the end of the experimental period, the mice were euthanized, and the brain, kidneys, lungs, heart, and spleen were dissected, homogenized, and analyzed spectrophotometrically for acetylcholinesterase activity. The results demonstrated that 14-day exposure to PS-MPs caused a statistically significant increase in AChE activity in the brain and a decrease in the lungs of both sexes compared to the control group. In the spleen, AChE activity decreased in females, whereas in the kidneys it decreased in males. Higher AChE activity values were observed in the lungs and kidneys of female mice compared with males. In conclusion, these findings indicate that short-term exposure to PS-MPs alters AChE activity in multiple organs. Such disturbances in AChE activity, whether inhibitory or stimulatory, may contribute to neurological, muscular, and systemic pathologies. Further research is required to elucidate the mechanisms by which microplastics affect organ and tissue function.

Key words: polystyrene microplastics, acetylcholinesterase activity, mice
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DISTRIBUTION OF ICG DYE IN RODENTS WHEN ADMINISTERED INTRANASALLY, INTRAPERITONEALLY, OR INTRAVENOUSLY

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This study aims to clarify the differences in the distribution of Indocyanine Green (ICG) in rodents when administered intranasally, intraperitoneally, or intravenously. ICG is a water-soluble tricarbocyanine dye widely used in biomedical research for various medical and scientific purposes. It fluoresces in the near-infrared spectrum, enabling deep tissue visualization. Owing to these properties, the distribution of the dye in rodents can be tracked across different organs, both *in vitro* and *in vivo*, using a near-infrared imaging system.
Intranasal application of ICG directs the dye toward different brain regions, as demonstrated in our recent publication (Mishonova et al., 2025). Following administration, a portion of the dye distributes across multiple organs and tissues, including the liver, esophagus, and digestive system. After injection, ICG circulates in the bloodstream, binds to plasma proteins, and is selectively cleared by the liver. At different time intervals, distinct organs exhibited fluorescence, indicating a time-dependent distribution of the dye. When ICG is injected intraperitoneally, fluorescence is observed in the abdominal fat and other adipose tissues, including the fat surrounding various organs which was not observed with intravenous administration.
Conclusion: When ICG is administered intravenous, it crosses the blood–brain barrier, reaching the brain and becoming diffusely distributed in brain tissue. When administered intranasally, it reaches the brain through different pathways and ICG is specifically distributed in the olfactory bulbs and brainstem. However, when administered intravenously, the dye is taken up by hepatocytes from the bloodstream and excreted into the bile, which then flows into the intestines, where it is eventually eliminated from the body reducing the amount of dye that reaches the brain. In all cases, fluorescence varied over time, with different organs showing distinct signal intensities at various time intervals.
Mishonova Milena, Lea Koceva, Bissera Pilicheva, Plamen Zagorchev, Petar Eftimov, Hristo Gagov, Iliyana Sazdova. Nose-to-Brain Delivery of Indocyanine Green in Rats. *C. R. Acad. Bulg. Sci.*, 78(10), 1456–1462, Oct. 2025.

Scientific session 5:

NEUROANATOMY, NEUROPHARMACOLOGY

NANOPARTICLES IN THE THERAPY OF NEURONAL DISEASES

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Drug delivery nanoparticles offer several advantages in the treatment, diagnosis, and management of neuronal diseases such as Alzheimer's, Parkinson's, multiple sclerosis, etc. They could provide selective delivery of drugs to affected brain regions, enhanced transport through the blood–brain barrier and sustained release of the drug. All these processes lead to reduction of systemic toxicity. In addition, encapsulation of unstable drugs in nanoparticles could protect them from environmental factors. In particular, the encapsulation of labile neurotherapeutic agents (e.g. some small molecules, proteins, nucleic acids) protects them from degradation provoked by enzymes, pH, light etc. Some nanoparticles combine diagnostic and therapeutic functions that enable real-time monitoring of treatment efficacy, e.g. gold nanoparticles can be used for optical imaging or photothermal therapy. In some cases, the nanoparticle carriers could augment the neuroprotective or antioxidant properties of the loaded drug, e. g. such effect was registered for nanoparticles based on chitosan.
This presentation will highlight recent achievements in nanotechnology related to nanosized drug delivery systems. Various types of nanoparticles will be presented e.g. polymeric nanospheres, nanogels, micelles, liposomes etc. The main advantages and challenges of these nanosystems will be discussed. The opportunity to improve the effect of some molecules with natural origin will be also exampled.
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THE NATURAL POLYPHENOL RESVERATROL AS A POTENTIAL NEUROPROTECTOR

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Resveratrol is a natural polyphenol, in particular stilbenoid, which can be found in peanuts, grape skin, raspberries, blueberries, red wine, etc. It possesses antioxidant, vasorelaxant and anti-inflammatory activities which are a prerequisite for potential neuroprotective action. Various enzymes, such as monoamine oxidase (MAO), acetylcholinesterase (AchE) and cyclooxygenase-2 (COX-2), play a pivotal role in the onset and progression of neurological disorders such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis. The inhibition of acetylcholinesterase for example leads to increased levels of acetylcholine and alleviation of Parkinson's disease symptoms. The MAO-B enzyme leads to degradation of dopamine, GABA and hydrogen peroxide production, related to mitochondrial dysfunction, while COX-2 is responsible for neuroinflammation. Therefore, the inhibition of these enzymes could be beneficial for the treatment of neurological diseases, too. However, resveratrol possesses limited aqueous solubility which requires application of technological approaches aiming to enhance its bioavailability and pharmacological effects. Encapsulation in nanosized drug delivery systems could be considered an appropriate strategy for coping with this issue. Taking this into consideration, the possible inhibitory effect of resveratrol on these enzymes was evaluated via molecular docking utilizing CB-Dock2 which is based on AutoDock Vina docking. Resveratrol (CID 445154) was used as a ligand, while MAO-B (1GOS), AchE (4PQE), and COX-2 (4RRW) were used as receptors. Resveratrol showed sufficient binding potential regarding all enzymes. The highest binding activity was detected for MAO-B (-8.3), followed by AchE (-8.0) and COX-2 (-7.6). Thus, these preliminary results are a foundation for further in vitro and in vivo research on the potential enzyme inhibitory effect of resveratrol. In order to enhance the solubility and bioavailability of resveratrol, encapsulation in Pluronic P123-Pluronic F127 micelles and in bovine serum albumin nanoparticles was performed. The micelles were characterized with 89.4% encapsulation efficiency and approx. 33 nm mean diameter, while the albumin nanoparticles showed 76.2% encapsulation efficiency and 145 nm mean size. Increased dissolution in comparison with the non-loaded resveratrol was observed for both formulations.

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ERYTHROCYTES, GRANULAR BODIES AND MICROBIOME

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Erythrocyte inclusions are granular structures that are observed in the erythrocytes of sick and healthy individuals. Such are Howell-Jolly bodies, reticulocytes, Cabot rings, Pappenheimer bodies, ribosomal fragments, Heinz bodies, etc. Of the erythrocyte granular structures, Heinz bodies are of particular interest, discovered and described by Robert Heinz. It is believed that Heinz bodies appear during oxidation of the sulfhydryl groups (S-H) in hemoglobin, which results in hemoglobin precipitates located peripherally on the erythrocyte membrane. Studies have shown that some antioxidants can reduce the formation of Heinz bodies. Some drugs, such as paracetamol, synthetic vitamin K1, phenothiazine, sulfacetamide, etc., can also cause the formation of Heinz bodies.

Conversely, the erythrocyte microbiome acts as a vital indicator of blood health. Indirect evidence from radiometric studies further corroborates the existence of living microbial entities within erythrocytes. Our research indicates that the microbiota present in the bloodstream features a distinct cell wall and reproduces via budding or cell wall shedding processes. When subjected to stress conditions of 43°C for a period of 24 hours, the blood microbiota proliferate as microbial cells devoid of a cell wall, leading to the emergence of electron-dense or electron-bright bodies. These electron-dense entities reproduce through fission, producing Gram-negative daughter cells, or by enlarging until they burst, thereby releasing daughter bodies. Furthermore, we have shown that the blood microbiome includes a considerable diversity of bacterial species. We quantitatively assessed the culturable fraction of blood microbiota in healthy individuals by culturing freshly drawn blood in Brain Heart Infusion medium enriched with 10% sucrose and a high concentration of Vitamin K (1 mg/mL), incubated at 43°C for 24 hours. The rapid proliferation of microbial structures was observed through light microscopy on Gram-stained slides. To differentiate the culturable fraction of the blood microbiota, we employed targeted sequencing of 16S rDNA and internal transcribed spacer markers.

In summary, our research addresses a significant knowledge gap and offers an analysis of the cultivability of circulating blood microbiota. Our results demonstrate a high level of biodiversity and unique mechanisms of propagation for the microbiota present in the blood.

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