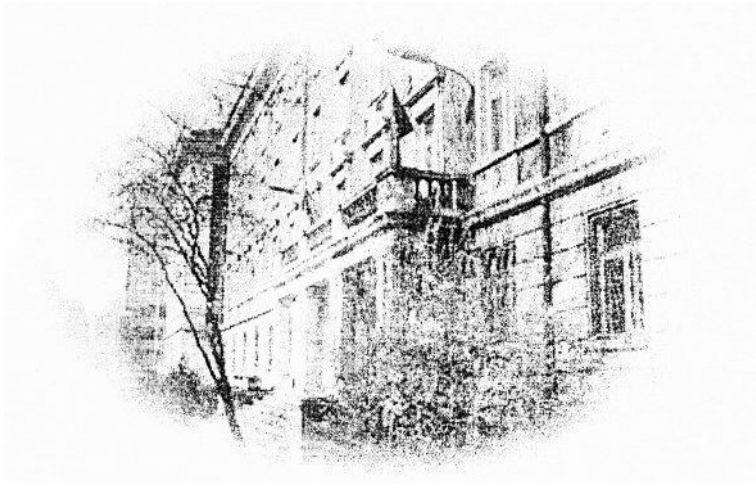




**NATIONAL SCIENTIFIC CONFERENCE
“75 YEARS INSTITUTE OF NEUROBIOLOGY”**

**DECEMBER 15-16, 2022
Sofia**



BOOK OF ABSTRACTS

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ORAL PRESENTATIONS

ESTIMATING DIRECTIONAL CONNECTIVITY IN CONTEMPLATIVE BRAIN STATES USING GRANGER CAUSALITY

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Background. Our previous work has demonstrated that long-term practice of contemplative brain states leads to a pronounced increase in brain connectivity (Yordanova et al., 2020, 2021). Using quantifiers of unidirectional functional connectivity has revealed that in highly experienced practitioners, the connectivity is increased in the left hemisphere with a posterior integrating focus and in the right hemisphere with a parieto-occipital integrating focus. While these observations reveal important changes in brain connections as a result of meditative training, it remains unclarified how the direction of information flow is affected by long-term contemplative practice.

Objective. To evaluate the effects of long-term contemplative practice on directional (effective) connectivity.

Methods. Electroencephalogram (EEG) was recorded at 64 electrodes in 22 experienced meditators (MED) and 17 controls (CON) for 6 min while they sustained states of rest, focused attention meditation (FAM), open monitoring meditation (OMM), and loving-kindness meditation (LKM), in which attentional focus had to be maintained in different ways. Directional connectivity was assessed by computing Granger causality (GC) as a time domain approach. GC is based on the assumption that if a signal can be predicted by the past information from a second signal better than the past information from its own signal, then the second signal can be considered causal to the first signal. To capture pairwise causal relations, the pairwise-conditional GC was used rather than the alternative multivariate CG that tends to distribute the causal effects across too many sources. CG was computed for 4 clusters combining electrodes in the left-frontal (C1), right-frontal (C2), left-posterior (C3), and right-posterior (C4) hemispheres.

Results. In the four conditions (Rest, FAM, OMM, and LKM), the dominating connectivity pattern in CON was characterized by the pronounced bi-directed inter-connections between the two posterior clusters C3 and C4. However, the major connectivity pattern in MED was dominated by the increased information inflow to the two frontal clusters, C1 and C2. Also, the increased information inflow to the frontal clusters in MED, as compared to CON, was particularly

strongly supported by an enhanced outflow from C1 to C2 and by enhanced outflow from posterior to anterior clusters, especially from C4 to C1. The main patterns of directed connectivity were stable across all conditions (Rest, FAM, OMM, and LKM) in both CON and MED.

Conclusion. These results show for the first time that long-term meditation practice is associated with an increased flow of information from posterior to frontal cortical regions as well as with increased directed connectivity from the left to the right frontal cortex.

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CONNECTIVITY OF MOTOR NETWORKS REVEALS NOVEL ASPECTS OF ERROR PROCESSING IN THE BRAIN

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Background. During motor responses, the activation of the motor cortical regions emerges in close association with the activation of the medial frontal cortex implicated with performance monitoring and cognitive control. There is evidence that during all phases of movement generation (preparation, initiation, and execution) motor regions are functionally coupled with performance monitoring/cognitive control regions (Urbano et al., 1998; Cavanagh et al., 2009; Kolev et al., 2022).

Objective. The present study analysed the connectivity of oscillatory response-related potentials during correct and error responses to explore whether and how motor oscillations during performance errors might be modified by a continuous communication between performance monitoring and movement generation regions.

Methods. Response-related potentials (RRPs) of correct and error responses were recorded at 64 electrodes and analysed in a sample of young adults while they performed a four-choice reaction task with the fingers of the two hands. RRPs were decomposed in the time-frequency (TF) domain using continuous wavelet transform and were spatially enhanced by means of current source density (CSD). Delta (1–3 Hz) and theta (3.5–7 Hz) TF components at extended motor areas contra- and ipsi-lateral to the responding hand were analysed with respect to functional connectivity. Functional connectivity was evaluated using

the phase-locking value (PLV), capturing the phase synchronization between pairs of electrodes. Two relevant parameters were computed: (1) the functional connectivity of motor regions with the medial frontal cortex (FCz-PLV), and (2) the connectedness of motor cortical areas with all other cortical regions (R-PLV).

Results. Errors were accompanied by prominent modulations in motor oscillations. (1) Although both theta and delta FCz-guided oscillations were most strongly synchronized at sensorimotor regions contra-lateral to the response, only theta FCz-PLV was significantly reduced by errors. (2) The connectedness of motor regions with all other cortical areas was significantly stronger for the hemisphere contra-lateral to the response for both delta and theta oscillations. However, only the connectedness of contra-lateral motor regions supported by delta networks was substantially reduced during errors. Notably, this reduction started as early as 200 ms before the error.

Conclusion. These observations indicate that the electrophysiological signatures of performance errors are not limited to the medial frontal signal Error Negativity, but they also involve the connectivity of oscillatory motor networks. Changes in motor cortex connectivity may represent early markers of emerging errors.

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LOWER LIMBS BILATERAL ASYMMETRY OF KNEE EXTENSORS IN HEALTHY MEN

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The initial length of the muscle, which is dependent on the angular position of the joint, is the major determinant of the generated force moment. When measuring knee bilateral strength differences in healthy untrained people, the dominant limb (DL) is considered to be stronger or equal to the non-dominant limb (NDL). The main concern about this fact is the low number of tested positions, which usually involve only parts of the knee range of motion. To the best of our knowledge, there are a limited number of studies that test the whole range of motion in the knee and give a complete understanding of the changes in force moment between the DL and NDL in healthy people. Testing a wide

range of angle positions gives us the opportunity to generate torque-angle curves, which provides a way of examining the average and personal curve tendencies of the bilateral asymmetries in healthy people. In our opinion, an individual approach to the variations of the optimal muscle length for maximal strength generation would open important insights into the bilateral asymmetry origin rather than the averaged values. Our aim is to generate and analyze the individual torque-angle curves and bilateral asymmetry indexes (BAI) of the knee extensors in the DL and the NDL in healthy untrained people.

Isometric peak torques were measured with isokinetic dynamometry (Biodex 4Pro) in 12 healthy untrained men (age 26 ± 7 y., height 1.8 ± 0.1 m, body mass 81 ± 11 kg) in positions ($^{\circ}$): 20, 40, 60, 70, 80, 90, 100 and 110. The torque-angle relationships were modeled by rational functions curve fitting. The BAI was calculated as the percentage difference between the stronger and the weaker leg. The individual analysis of the torque-angle relationships divided the subjects into three strength profiles regarding the BAI. The first torque-angle curve type is observed in 25% of the subjects and presents insignificant strength differences between the extensors of the DL and the NDL in the whole tested range of motion and BAI below 15%, which is an expected result for healthy untrained people. In the second group (42% of the participants), significant differences were observed between the extensors of both legs mainly in the range of 80-110 $^{\circ}$ of knee flexion. Through this range of motion, where the muscles that execute the extension are lengthened, the DL generates higher torque values compared to the NDL. At these positions, the BAI range from 20% in some subjects up to 40% in others, which is considered a risk factor for injury. The third curve type (33% of the participants) exhibits a curve pattern in which the NDL generates higher torque values in the range from 20 to 70 $^{\circ}$ knee flexion (where the extensors are shorter), but from 80 to 110 $^{\circ}$ both limbs switch positions and the DL shows significantly higher values, compared to the NDL. Our results are similar to those available in the literature for 90 $^{\circ}$ knee flexion, where the dominant limb is stronger, but as is shown, the strength profile changes throughout the range of motion. These findings on the types of bilateral asymmetry profiling of healthy people provide a more specific basis when using healthy people as controls.

In the present study, it has been shown that there are three main types of torque-angle curves when evaluating the bilateral isometric knee extensor strength in healthy untrained men. The types of torque-angle curves are: -DL and NDL with no strength differences; -stronger DL only at 80 to 110 $^{\circ}$; -stronger NDL at 20-70 $^{\circ}$, but lower at 80-110 $^{\circ}$. The individual approach to analyzing bilateral torque-angle curves provides a wider and more complex understanding of the biomechanical nature of the strength asymmetries in healthy people.

AN INVESTIGATION OF VOLUNTARY SACCADIC EYE MOVEMENTS DURING THE PRESENTATION OF VISUAL IMAGES IN DIFFERENT PARTS OF THE VISUAL FIELD

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The present work provides us with initial data - pilot studies on influencing the characteristics of volitional saccades after the immediate application of semantic priming related to the expansion of the focus of spatial attention (Bargh, Chen, & Burrows, 1996), (Bock et al. 1. 2013), (Staneva et al. 1, 2019). Five healthy persons aged 26 and 50-55 years were examined. Eye movement recording distance was 60 cm from a 27-inch screen with a resolution of 768 x 1366 pixels. The head of the examined person was fixed with the help of a chin rest. Eye movement recordings were made using an Eye Tracker GP3HD at 150 Hz. Licensed products were used to calibrate and record eye movements. Additional software was developed to the licensed software of the device in the Python programming language to register the parameters (total duration of all fixations from the start of the recording in seconds; duration of single fixations in seconds; saccade magnitude, saccade direction. The visual scene was divided into 15 equal-sized squares of 6.4 X 6.4 cm. In each of them, in a pre-defined order, appeared a target replaced by an image after a fixed delay. After a delay of 3 s, four other targets appeared in the upper, lower, left, and right squares at a distance of 6.4 cm from the screen center and were replaced by four images after 3 s. The first session of the experiment involved observing the images that appeared on the screen, in the second session the subjects completed a test, applying semantic priming. The repeated measures ANOVA was applied to the data. The results of the analysis show a significant effect of priming on the latency of the first saccade ANOVA ($F(1, 697)=1408.7$, $p<0.0001$). The saccade magnitude and direction also show a significant effect of the priming after a logarithmic transformation $F(1, 995) = 141.358$ $p<0.00$. The results also revealed a significant effect of the participant's age and interaction between the age and the latency of the first saccade and the fixation duration $F(3, 466.41)=70.12$ $p< 0.001$.

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NEURODEGENERATION, OXIDATIVE STRESS, AND IRON

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A number of studies have shown a link between iron accumulation in the brain, oxidative stress, and the pathogenesis of various neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, Huntington's chorea, Amyotrophic Lateral Sclerosis, and Neurodegeneration with Brain Iron Accumulation). Ferroptosis, an iron-dependent programmed cell death, is currently hypothesized to be the primary driver of neurological cell death. The possible underlying mechanism of ferroptosis is the participation of ferrous iron in the generation of hydroxyl radicals and alkyl radicals with subsequent oxidative damage to cellular structures. This hypothesis is supported by the evidence that ferroptosis can be prevented by lipophilic antioxidants, such as vitamin E, and by iron chelators, including deferoxamine. Thus, the attention is focused on therapeutic strategies for metal-chelating drugs. In our laboratory's long-term work, several substances with antioxidant activity, incl. metal chelation, and potential for neuroprotective effect have been studied: lipoic acid, ellagic acid, myrtenal, garden snail (*Cornu aspersum*) mucus extract, etc. The effects of these natural products were tested on a rat model of Alzheimer's disease induced by Scopolamine (Sco). The results have shown significant prevention and suppression of brain oxidative stress by the tested substances. In the brains of Sco-treated rats, the snail mucus extract reduced lipid peroxidation (LPO), restored the superoxide dismutase (SOD) activity, and significantly recovered the glutathione (GSH) levels. The treatment of dementia animals with lipoic acid led to reduced LPO levels, increased GSH, restored catalase (CAT) activity, and significantly decreased glutathione peroxidase (GPx) activity. The ellagic acid restored LPO levels, CAT, and SOD activity in the brains of rats injected with Sco. The myrtenal intake by the Sco-treated rats led to decreased brain LPO by 19%, increased GSH content by 14.8%, and restored SOD activity to the control level in comparison to those of the Sco-treated rats. However, treatments of patients with neurodegenerative diseases by antioxidants and iron-chelating agents have not demonstrated a compelling clinical effect. In regards to iron, it is assumed that these failures may be due to a lack of specificity leading to the

depletion of iron stores and other essential metals required for maintaining neuronal health. Obviously, future research is needed to define and regulate iron homeostasis in the brain.

CAN SEMINAL PLASMA BE A RESPONSIBLE INDICATOR OF FERTILITY?

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Seminal plasma (SP) is the non-cellular component of sperm. It has a heterogeneous composition comprising secretions of the testis, epididymis, and accessory gonads. Its composition, despite species specificities, contains specific hormones and proteins, including cytokines and enzymes, cholesterol, DNA and RNA. Nucleic acids are often protected in extracellular vesicles derived from the epididymis or prostate. This issue raises the question of whether SP is involved in various events in male gametes before fertilization. SP also interacts with the multiple compartments of the tubular genital tract, causing changes in gene function that prepare for an eventual successful pregnancy. After all, does it modulate fertility in this way?

These questionable concepts arise because spermatozoa (epididymal or washed ejaculated) without SP are still fertile. So this report aims to distinguish between the in vivo role of SP after sperm release in the female tract and the effect of SP on sperm treated for artificial reproduction.

This review attempts to summarize the current knowledge of the reproductive roles played by SP components in the human species, which is increasingly affected by infertility, as well as our results in animal models.

SEX-DEPENDENT EFFECT OF CHRONIC PIROMELATINE TREATMENT ON PRENATAL STRESS-INDUCED MEMORY DEFICITS IN RATS

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Prenatal stress impaired cognitive function in rats, while Piromelatine treatment corrected memory decline in male rats with chronic mild stress. In the present study, we aimed to evaluate the effect of chronic treatment with the melatonin analogue Piromelatine on associative and spatial hippocampus-dependent memory in male and female offspring with a history of prenatal stress (PNS). We report that both male and female young adult offspring with PNS treated with vehicle exhibited reduced memory response in the object recognition test (ORT), while cognitive performance in the radial arm maze test was worsened only in the male offspring. The 21-day treatment with Piromelatine of male and female offspring with PNS attenuated the impaired associative memory in the two sexes and the impaired spatial memory in male offspring. There was no effect of prenatal stress on plasma melatonin levels in male and female rats. Piromelatine treatment in male offspring with PNS tended to elevate the release of melatonin in plasma compared to the offspring treated with the vehicle but was unable to reach a significant difference. This melatonin compound did not influence the hormonal release in female PNS rats. Our results confirm the sexually dichotomous potential favorable effect of this melatonin analogue in pathological conditions related to memory impairment.

E-POSTER PRESENTATIONS

FUNCTIONAL CONNECTIVITY IN CHILDREN WITH DEVELOPMENTAL DYSLEXIA DURING READING

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A phonological deficit, which implies a deficit in phonological awareness, followed by a visual deficit associated with poor spelling due to poor coding of letter positions or an ineffective functional neural network, suggests an overall inefficiency of information processing and underlies developmental dyslexia (DD). Knowing the performance of the reading network is useful for understanding reading difficulties. The electroencephalographic (EEG) studies, using graph theoretical analysis, have found functional connectivity abnormalities in dyslexics. How visual nonverbal training (VT) and neurofeedback training (NF) can change the functional connectivity of the reading network in developmental dyslexia is still unclear.

The hypothesis is that brain training of children with developmental dyslexia should increase the efficiency of reading circuits by improving short-range connections in the left hemisphere, which has not yet been established as dominant.

The graph method of the minimum spanning tree (MST) was used to construct the reading networks in multiple EEG frequency bands. The functional connectivity in the reading neural network was investigated by comparisons of the local and global topological properties of functional reading networks between controls and children with DD before and after training (NF-VT) during a reading of words.

Compared to a control group of typical-reading children, children with DD had a less segregated neural network (global MST measures: a higher leaf fraction, tree hierarchy, kappa, and smaller diameter) before training in θ - γ frequency bands. After training, significant differences were found in the global MST measures between children with DD before and after training in the θ , β , and γ frequency bands, which revealed a more segregated network topology of the children with dyslexia close to those of the controls.

In β 1 and γ -frequency bands, pre-training dyslexics exhibited changes in the local MST measures as a reduced degree and betweenness centrality of hubs in superior, middle, and inferior frontal areas in both brain hemispheres compared to the controls. Dyslexics relied on the left anterior temporal (β 1, γ 1) and dorsolateral prefrontal cortex (γ 1), while in the right hemisphere, they relied on

the occipitotemporal, parietal, ($\beta 1$), motor ($\beta 2$, $\gamma 1$), and somatosensory cortices ($\gamma 1$).

After training, hubs appeared in both hemispheres at the middle occipital (β), parietal ($\beta 1$), somatosensory ($\gamma 1$), and dorsolateral prefrontal cortices ($\gamma 2$), while in the left hemisphere, they appeared at the middle temporal, motor ($\beta 1$), intermediate ($\gamma 2$), and inferior frontal cortices ($\gamma 1$, $\beta 2$). Language-related brain regions were more active after the NF-VT training. They contribute to an understanding of lexical and sublexical representation. The same role has areas important for the articulatory processes of reading.

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DIFFERENTIAL ASSOCIATIONS OF MENTAL STRESS ORIGIN WITH NEUROCOGNITIVE FUNCTIONS

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Background. Environmental stressors are recognized as a high-risk factor for developing mental stress as well as deviations in a variety of physiological systems. The continuous accumulation of stressful events appears a particularly high-risk factor. Importantly, the subjective experience of stress may not directly reflect the amount of environmental stressors. In addition to life stress events, contemporary working conditions pose high demands to individual capacity and may also lead to mental stress, known as burnout syndrome. Irrespective of its origin, chronic stress affects the regulation of nervous system processes and is a high-risk factor for problems in a variety of physiological, immune, and metabolic circuits. Notably, although the effects of stress on cognitive functioning are recognized by the overall decreased capacity of the individual to sustain high working and quality-of-life performance, the sensitivity of specific cognitive operations to stress is less well understood.

Objective. To explore the effects of stress on several neuro-cognitive functions linked to cognitive control: working memory capacity, inhibitory control, and interference control.

Methodology. A total of 109 participants with a high-risk professional occupation were enrolled. To assess the life-stressors load, the Holmes Rahe

stress scale was applied. Working stress was evaluated using the Oldenburg Burnout Inventory (OLBI). To control for the effects of possible latent depression that may accompany high stress/low resilience dispositions, the Patient Health Questionnaire depression scale (PHQ-9) also was used. Working memory was tested by means of a 2-back task. The Kopp version of the Flanker task was employed to study inhibitory control, and the Stroop task was used to reflect interference control. To test the associations of neurocognitive functions with mental stress, multiple regression stepwise analyses were used.

Results. Although the three cognitive functions under study were all guided by the cognitive control system encompassing attention, they were differentially predicted by stress-related scores. Notably, the accumulation of stressful life events predicted an accentuated increase in inhibitory reactions. An emerging deficit in interference control was predicted by burnout scores, whereas a decrease in working memory capacity was associated with latent depression.

Conclusions. The present results demonstrate that mental stress affects neurocognitive functioning in selective ways rather than leading to deteriorations in a unitary manner. They provide novel evidence for the specific responsiveness of working memory, inhibition, and interference control to environmental mental stress, working stress, and latent depression.

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GENDER-SPECIFIC DIFFERENCES IN AUDITORY SELECTIVE ATTENTION IN THE COURSE OF DEVELOPMENT: AN ERP STUDY

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Background. Differences between males and females in cognitive abilities at the behavioral level are traditionally thought to be due to differences in cognitive strategies between the two genders (Steffensen et al., 2008; Choi et al., 2015; Hirnstein et al., 2019; Kasai, 2021). Developmental research on this issue is very scarce (Bakos et al., 2016).

Objective. The aim of the present work was to investigate gender-specific neurophysiological processes of selective attention in children and adolescents.

Method. An auditory selective attention task was used. Two stimulus types with different probabilities (40% for targets and 60% for non-targets) were presented randomly to the right and left ear. During the task participants had to maintain lateralized internal attention either to right- or left-side stimuli in order to produce selective motor responses with the right hand to targets appearing in the attended side. Thus, there were two conditions - attend-right and attend-left, each comprising 4 stimulus types - target-attended, non-target-attended, target-non-attended, non-target-non-attended. An electroencephalogram (EEG) was recorded at 10 electrodes during task performance. Reaction times to targets and components of event-related potentials (ERPs) were analyzed in 110 healthy, right-handed children and adolescents 9-16-years-old, divided into four age and two gender groups (age: 9-10, 11-12, 13-14, and 15-16-years-old; gender: female, male). Effects of age, gender, laterality (left, right), region (frontal, central, parietal), attention (non-attend, attend), side of stimulation (left, right), and stimulus type (non-target, target) were explored.

Results. *Early processing:* A gender difference was observed for the non-attended stimuli. In girls, these stimuli evoked larger N1P2 amplitudes at left-vs. right hemisphere electrodes, whereas in boys, these inter-hemispheric differences were not expressed at all regions. A gender difference also was observed for non-target stimuli, which evoked overall greater P2N2 amplitudes in girls as compared to boys. Furthermore, P2N2 amplitudes were larger for non-targets vs. targets only in girls, in contrast to boys. *Late processing:* Gender differences were observed for attended stimuli. N2P3 amplitude of girls was larger for attended vs. non-attended stimuli. In contrast, N2P3 amplitude, including critically the parietal areas, was not affected by attention in boys. These gender effects persisted throughout the age period under study (9-16 years).

Conclusions. The ERPs analysis revealed that during auditory selective attention, the early processing of both the non-attended and non-target stimuli was augmented in girls. In addition, the late processing of attended stimuli was further emphasized in girls as compared to boys. These results demonstrate that in childhood and adolescence (9-16 years), gender is an efficient factor modulating the neurocognitive strategies during auditory selective attention such that females process more efficiently task-irrelevant information at early attentional selection and task-relevant information at later stages of cognitive evaluation.

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EYE MOVEMENTS OF CHILDREN AND ADOLESCENTS WITH ASD, ADHD AND DD DURING DIFFERENT VISUAL PERCEPTION TASKS

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Background: Atypical eye movements have been described in developmental disorders such as Autism Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD), and Developmental Dyslexia (DD). Usually, the eye movement characteristics of these disorders are studied under different conditions and tasks that substantially complicate the comparison between the disorders.

Objective: The present study aimed to register the eye movements in groups of participants with ASD, ADHD, and DD during three visual perception tasks and to compare them to the control group with typical development (TD).

Methods: Eighty children and adolescents from four groups: with ASD, ADHD, DD, or TD participated in three visual perception tasks: contour integration, global motion discrimination, and reading words or pseudowords. In each task, the eye movements of all the participants were recorded and analyzed later.

Results: The data obtained showed larger intra- and interindividual differences in fixation stability for all the groups with developmental disorders: ASD, ADHD, and DD, compared to the TD group. The fixation instability was larger in the ADHD group during the contour integration task, while the most significant individual differences and the highest individual fixation variability were found for the ASD group. During the global motion discrimination task, participants with ASD had significantly more fixations with diminished duration in high external noise condition. A tendency for more fixations with shorter duration was also found in groups with ADHD and DD compared to the control group. In addition, the saccade amplitude was the largest for the group with ASD. During reading both words and pseudowords independently of the noise level and the length of the letter string, the group with ASD showed the tendency for the longest but unstable fixations. The amplitude of the saccades was the largest in the ASD group and the smallest – in the TD group.

Conclusions: The eye movement characteristics in the separate visual perception tasks allow differentiating the participants with various neurodevelopmental disorders. The group that deviates most is the ASD group which shows the largest saccade amplitude in connection with the task and fixation instability in tasks of varying complexity and uncertainty levels. These features indicate a different way of deriving and processing visual information.

EFFECTS OF SPORT EXPERIENCE ON THE VISIO MOTOR REACTION TIMES IN BOXERS

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Background: Adequate evaluation of the situation and timely reactions are crucial for performance in sports such as box. There are few studies on the visio-motor reaction times of boxers.

Aim of study: Evaluation of the visio-motor reaction times of boxers during a complex dynamic cognitive task.

Methods: Sixteen boxers (mean age, all right-handed males) participated in the study. They were divided in two groups according to their boxing experience: 1-4 years and more than 5 years. From a standardized standing position, they were instructed to press as fast as possible buttons with their dominant and non-dominant hand, depending on the colour of the button. The buttons were installed on the wall at eye-level and flashed red or green in random order and position. Each person had the same sequence of 48 stimuli. Trials above 1000 ms and errors (wrong hand) were not included in the data analysis. Hits were analyzed as “jab” (forward) or “cross” (diagonal) according to the position of the button and the required arm movement.

Results: The visio-motor reaction times of the group with more than 5 years of experience were shorter than the first group. The “jab” hits were faster than the “cross” ones in both groups. Sport experience had a greater effect on the “jab” reaction times than the “cross” ones. In both groups of boxers, the visio-motor reaction times with the dominant hand were shorter than the non-dominant.

Conclusion: Due to the specific training of boxers, their automatic reaction abilities during task-specific cognitive load increase more prominently in the “jab” hits. Despite sport experience, the effects of hand laterality on the visio-motor reaction times is preserved.

FACTORIAL STRUCTURE OF THE BULGARIAN VERSIONS OFB PHQ-0 AND OLB1

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Psychometric tests are widely used in almost any area of psychology. However, the proper application and interpretation of such instruments are contingent on the extent to which the results comply with the underlying theoretical assumptions of the test, i.e., its construct validity. It is, therefore important to run validation studies that aim to verify that test scores from a particular population conform to the structure of the test. Validation studies not only assure the correct interpretation of test results but can also contribute by discovering the limitations of the test and generating new theoretical insights.

Here we present ongoing work on establishing the validity of the Bulgarian versions of two psychometric tests - the Patient Health Questionnaire (PHQ-9) and the Oldenburg Burnout Inventory (OLBI). PHQ-9 consists of 9 items for the assessment of depression, and OLBI has 16 items for evaluating working stress (burnout). Data collection was performed online and resulted in a sample of 106 participants, each completing both tests. The scores were analyzed by using several statistical tools which address various validation aspects. In particular, we demonstrate how confirmatory factor analysis (CFA) can be used to gain insight into the latent factor structure by evaluating and comparing alternative models. We also show how CFA can be complemented with exploratory factor analysis (EFA) in order to resolve apparent discrepancies in the data. Altogether, the results of our analysis support the proposed structure of PHQ-9, but also imply that the theoretical assumptions of OLBI need to be adjusted in order to be able to account for the population we studied.

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PERFORMANCE MARKERS OF EXPLICIT KNOWLEDGE GENERATION DURING IMPLICIT LEARNING

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Background. When people are exposed to environmental regularities, only some of them are able to consciously discover these regularities. Importantly, if people acquire such explicit knowledge (ExK), they can substantially enhance their achievements by deliberately applying analytic and explorative cognitive strategies. It is, however, not well known which individual qualities, in terms of cognitive or neurophysiologic dispositions, facilitate bringing knowledge to awareness. To study experimentally such individual faculties, tasks with dual structure (overt and covert) are typically used. In these tasks, a hidden rule is presented to participants (covert level) while their performance is guided by an instruction unrelated to this rule (overt level). Participants practice the task in implicit learning conditions where no information about the presence of a hidden rule is given. Major goals of such experiments are to explore the behaviour of those individuals who would be able to comprehend the hidden regularity.

Objective. Within this research perspective, here we present some performance characteristics of those individuals who are able to gain ExK about abstract regularities during implicit learning (explicit solvers, ES) that distinguish their behaviour from the behaviour of those who cannot gain ExK.

Methods. A visual serial response time task (SRTT) was practiced in implicit conditions by 109 participants. Unknown to the participants, a fixed sequence of 12 stimuli was delivered in blocks surrounded by blocks with randomly presented stimuli. The total number of structured stimuli during practice was 660 with 55 sequence repetitions. The following performance parameters were measured in ordered and random blocks: (1) Reaction time (RT), (2) Coefficient of variance (CV) of RT, (3) Error rate (commission and omission errors), (4) Implicit knowledge (ImK) coefficient reflecting the amount of acquired ImK about regularity (Yordanova et al., 2015), and (5) Number of correct premature responses (RT < 150 ms). ExK about the covert sequence was tested after a 10-hours long retention period.

Results. Out of 109 subjects, only 7 were able to gain ExK already during implicit practice (online ES), and 17 became explicit solvers after the retention (offline ES). Online ES manifested (a) a substantial RT decrease in regular blocks as reflected by the RT decrease and the large ImK coefficient, (b) a great increase in RT variance both in regular and early random blocks, (c) a decrease in error rate in regular blocks, and (d) a large number of premature responses in regular blocks. Offline ES (a) gained practice-based ImK at learning or after retention as reflected by the RT speeding in regular blocks and the large ImK coefficient, and (b) manifested a large RT variance at learning or after retention.

Conclusions. The performance patterns in explicit solvers, though appearing diverse, highlight performance variability as a crucial behavioural feature of those individuals who have the ability to bring hidden abstract knowledge to awareness. Notably, increased performance variability can be seen as a trait

feature in online ES since it characterized their performance irrespective of exposure to regularities. In contrast, performance variability was enhanced in offline ES as a result of the dynamics of ImK accumulation – before or after retention. Thus, two different sources of ExK generation can be proposed, one being based on an individual trait for active environmental exploration and the other depending primarily on the ability to acquire implicit knowledge about regularities.

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EFFECTS OF SNAIL *HELIX ASPERSA* EXTRACT ON MICROGLIA IN ALZHEIMER'S TYPE DEMENTIA

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Of interest is to investigate the effects of snail extract (SE) on microglia and macrophages in healthy rodents and in those with Alzheimer's type dementia. Microglia is formed during embryonic development. It has exceptional plasticity and maintains homeostasis in the brain. Microglia plays an important role in the response of the central nervous system to acute and chronic inflammatory processes. On the other hand, the role of macrophages is to engulf and digest cellular debris from pathogens. They are the body's professional phagocytic cells that attack all foreign substances, including microorganisms and tumor cells, destroying them and engulfing their remains.

We investigated the neurobiological activity of snail extract on healthy mice treated only with snail extract delivered by special food tube for 16 consecutive days and on those with Alzheimer's type dementia induced by intraperitoneal injection of scopolamine (Sco, 2 mg/kg) for 11 consecutive days. Snail extract (0.5 mL/100 g) was administered orally by food tube 5 days before the start of treatment with the neurotoxin scopolamine plus 11 consecutive days together

with scopolamine. Our results demonstrated that snail extract increased the amount of macrophages in the blood of both healthy mice and those with an experimental model of Alzheimer's type dementia. Snail extract treatment inhibited scopolamine-induced cluster of differentiation 68 (CD68) positive cells elevation in the hippocampal homogenate. Scopolamine slightly increased glial fibrillary acidic protein (GFAP) level in hippocampal tissue homogenate that was restored in the treated with snail extract group. GFAP is a key factor for astrocyte functions (regeneration and synaptic plasticity). We also observed that SE treatment inhibited Sco-induced P2RY12 protein expression in the hippocampus.

In conclusion, we might assume that the application of snail extract regulates microglial function by elevating the reparative phenotype of blood macrophages and reducing CD68+ cells, regulating P2RY12 and restoring GFAP expression in the hippocampus.

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EFFECTS OF SNAIL *HELIX ASPERSA* EXTRACT ON MOTOR PERFORMANCE AND MEMORY IN MPTP EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is characterized on the one hand by disorders in neuromuscular coordination (motor disorders) and, on the other, by non-motor disorders. It was called "tremulous palsy" by its discoverer, Dr. James Parkinson. Tremor, muscle stiffness, slowness of movements, and change in gait are among the first symptoms of Parkinson's disease. They are caused by impaired function of the basal ganglia nuclei and occur when striatal dopamine depletion is approximately 80 %. The non-motor features of Parkinson's disease, such as memory impairment, autonomic dysfunctions, and depression are due to the loss of neurons in the cholinergic, noradrenergic and serotonergic neurotransmitter systems. Currently, there are no strategies that can stop the brain cell injury in PD. Since snail extract (SE) possesses antioxidant, anti-inflammatory, and antiapoptotic properties, we studied its effects in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mice model of PD. Male mice (C57BL/6, male, 8 weeks old) were used for an experimental Parkinson's disease model via MPTP treatment. MPTP+SE (0.1 ml/10g b.w) group received standardized snail extract for 12 consecutive days. All animals were submitted to tests for motor coordination and memory on the 16th and 17th day. At the end of the experiment, the two main brain structures related to memory (prefrontal cortex and hippocampus) were separated for biochemical and histological analysis. Twelve days of SE treatment (7 days before and 5 days simultaneously with MPTP) was able to improve impaired motor and memory disturbance of the experimental animals with the MPTP model of PD. SE protected dopaminergic neurons, as can be seen in our biochemical studies. Twelve days after the first MPTP treatment, the reduction of brain DA was by 73% as compared to controls. Multiple SE administration increased DA brain levels (by 176 %) and decreased those of NA (by 42 %,) as compared to the MPTP group.

In conclusion, our results provide insight into the beneficial effects of *Helix aspersa* extract in an experimental PD model.

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**SPONTANEOUSLY HYPERTENSIVE RATS VS WISTAR KYOTO
AND WISTAR RATS: AN ASSESSMENT OF MEMORY
PERFORMANCE AND BDNF EXPRESSION IN THE HIPPOCAMPUS**

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Spontaneously hypertensive rats (SHR), are known as a model of essential hypertension and attention deficit hyperactivity disorder. In the present study, we aimed to ascertain the cognitive responses and their relationship with BDNF expression in the hippocampus of SHR, WKY, and Wistar rats. The SHR had impairment in associative and spatial working memory compared to Wistar but not WKY rats. Both naïve SHR and PTZ-injected SHR had low BDNF expression in the hippocampus, whereas a seizure-induced drop of this signaling molecule was observed in WKY and Wistar rats. Our previous and current results suggest that SHR might model some memory and epilepsy disorders related to low BDNF expression in the hippocampus.

SEX-DEPENDENT BEHAVIORAL CHANGES IN ADULT RAT OFFSPRING WITH PRENATAL MELATONIN DEFICIENT

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The prenatal environment significantly affects the development of the organism. Exposure of the fetus to adverse conditions has a serious deleterious effect on individual development with short- and long-term consequences for neuroendocrine and behavioral responses. Melatonin, being among the few hormones that cross the placental barrier without its molecule being changed, probably has an essential role in the regulation of the fetal circadian rhythm. The pineal gland of the fetus, which does not yet synthesize melatonin, is exposed to the mother's hormone. The aim of our study was to investigate the effect of melatonin treatment on pregnant rats exposed to a constant light regime on the behavioral responses of male and female sexually mature offspring. Melatonin was injected at a dose of 10 mg/kg, subcutaneously, from G0 to G21. Depressive-like behavior was assessed through sucrose preference test /SPT/ and forced swimming test /FST/ and cognitive functions through object recognition test /ORT/ on 60-day-old littermates of both sexes with a history of prenatal melatonin deficiency. Male and female offspring with prenatal melatonin deficiency were characterized by anhedonia /reduced preference for sweet solutions/ and increased immobility time in the FST test compared to matched control generations ($p < 0.05$). Depressive-like behavior of melatonin-deficient female offspring was corrected by prenatal melatonin treatment ($p < 0.05$). Memory deficit was reported in both sexes of rats with prenatal melatonin deficiency compared to matched controls ($p < 0.05$). Hormonal supplementation prevented disrupted associative memory in female offspring with prenatal melatonin deficit. In conclusion, our results suggest that melatonin supplementation has a gender-specific beneficial effect on behavioral changes resulted by prenatal melatonin deficit. Future studies are needed to ascertain the sex divergence in the efficacy of melatonin against depressive-like responses and memory impairment in the prenatal rat model.

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PRENATAL PROGESTERONE TREATMENT AFFECTS BEHAVIORAL RESPONSES IN ADULT RAT OFFSPRING IN A SEX-DEPENDENT MANNER

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Exogenous treatment during pregnancy with steroid hormones (estrogens, androgens, or glucocorticoids) affects the development of the fetus and the sexually mature generation. In clinical practice, the hormone progesterone is used therapeutically in programs for assisted reproduction, infertility treatment, threatened abortion, and premature birth. The hormone has a key role in establishing and maintaining pregnancy through its endocrine and immunological effects. The aim of the present study is to investigate sex-dependent changes in emotional status and memory responses of a generation of prenatally treated with progesterone offspring. Female pregnant rats were treated subcutaneously with progesterone (50 mg/kg) from gestational (G) period G0 to G10. Anxiety and depressive-like behaviour of male and female adult offspring was evaluated with an open field (OF) test, plus maze test (EPM), sucrose preference test (SPT), and forced swimming test (FST), while their cognitive responses were assessed through radial arm maze (RAM) test.

Prenatal treated with progesterone (PNP) male and female offspring exhibited lower horizontal and vertical activity compared to the male and female control rats in the open field test ($p < 0.05$). Male and female PNP offspring had elevated anxiety with decreased distance and time spent in the open arms compared to the matched controls in the EPM test ($p < 0.05$). The two sexes of PNP rats demonstrated depressive-like responses with anhedonia in the SPT and increased immobility time in the FST compared to the matched controls ($p < 0.05$). Prenatal hormonal treatment also worsened working memory in the RAM test in both sexes and increased the time needed to complete the task compared to controls. In conclusion, our results suggest that prenatal treatment with 50 mg/kg progesterone exerts a detrimental effect on emotional behaviour and memory abilities in both sexes. Future studies are needed to ascertain the underlying mechanism associated with these sustained behavioural abnormalities in adult offspring resulted by prenatal hormonal treatment.

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LOCAL EFFECTS OF NEWLY SYNTHESIZED HEMORPHIN ANALOGS ON AN EXPERIMENTAL MODEL OF HYPERALGESIA AND INFLAMMATION IN THE RAT

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Hemorphins are endogenously occurring peptides that are cleavage products of hemoglobin chains. They are classified as atypical opioids with antinociceptive effects that have been identified in various biological fluids and tissues, including the peripheral and central nervous systems. The synthesis of peptide analogs with substituted unnatural cyclic amino acids or an adamantane-based structure is a strategy to improve the propensity for aqueous solubility and/or to increase their affinity for the target receptor or enzyme. In the present study, we have analyzed the effects of three newly synthesized analogs of hemorphin with substituted cyclic amino acids and added adamantane residue on carrageenan-induced inflammatory hyperalgesia in rats.

The peptides were synthesized by solid-phase peptide synthesis (Fmoc-strategy) and injected intraplantar at doses of 25 and 50 $\mu\text{g}/5\ \mu\text{l}$ paw before the inflammation. We used an experimental model of inflammation induced by intraplantar injection of carrageenan, a "paw pressure" method to assess mechanical hyperalgesia, and plethysmometry to measure inflammatory edema. Indomethacin was used as the reference drug.

Local intraplantar injection of Ad5-V, Ad6-V, and Ad7-V produced an antihyperalgesic effect that lasted 4 h for the lower dose used and was less pronounced and of shorter duration at the higher dose. Ad5-V suppressed hyperalgesia at both doses throughout the observation period but provoked the most pronounced potentiation of carrageenan-induced edema. The other two peptides also potentiated paw edema compared with indomethacin and negative controls.

The results showed a significant and comparable to the reference drug antihyperalgesic effect of all three new molecules. The lower efficacy of the higher dose suggests more than one mechanism of action. The peptides potentiate the progression of carrageenan-induced paw edema, which excludes

anti-inflammatory activity as a potential mechanism of action. Further pharmacological studies should elucidate their mechanism of action against hyperalgesia with emphasis on the selectivity of the peptides to specific opioid receptors.

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GHRELIN IMMUNOREACTIVITY IN THE MYENTERIC PLEXUS OF THE RAT ANORECTAL REGION

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Ghrelin (Ghr) is a peptide hormone, mainly secreted by cells of the gastrointestinal tract, thought to regulate gut motility. The myenteric plexus (Auerbach plexus) is a group of ganglia that run throughout the entire gastrointestinal tract between the circular and the longitudinal layer of the tunica muscularis. The present report demonstrates the ghrelin immunoreactivity in the myenteric plexus of the rat anorectal region. By applying ghrelin immunohistochemistry, we observed intensely immunopositive structures with neural morphology between the circular and longitudinal muscle layers, identified as the myenteric ganglia. The ganglia were clearly delineated, the neuronal perikarya with an oval or polygonal shape. Discrete differences in staining intensity hint towards the presence of neurons with different expressions of ghrelin. We infer that ghrelin is a component of the brain-gut axis and modulates signals as a cotransmitter to the classical neurotransmitters and neuropeptides.

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STUDENT SESSION

MULTIPLE SCLEROSIS AND REPRODUCTIVE HEALTH

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Multiple sclerosis (MS) is a degenerative neurological disease with autoimmune pathophysiology and unknown etiology whose prevalence has increased worldwide in the past few years. This disease impairs patients' physical and psychological health as well as their social life, and thus it compromises the quality of life. The first symptoms of the disease appear most often in reproductive age (between the age of 20 and 40), as females are 2-3 times more likely to develop this disease rather than males. This raises concerns about family planning and reproductive health. The significant impact on these aspects of life indicates the need for discussion about the relations between MS and reproductive health – fertility, contraception, pregnancy, breastfeeding. There is no evidence that MS reduces the rate of successful pregnancy, although, according to epidemiologic studies, a significantly lower percentage of affected women become pregnant and give birth than healthy women. There is controversial data on clinical research on females with MS using a combined oral contraceptive, there is no data of the impact of progestin-only contraceptives on the disease. Sexual dysfunction is common in affected patients of both sexes, but it is often neglected by healthcare professionals. Research suggests implying an interdisciplinary approach to treating this condition. Furthermore, efficient treatment in family planning should be taken into consideration. The medical management of MS during pregnancy and the postpartum period is challenging because of the exposure of the foetus or infant. There is a prohibition on the use of some therapeutic agents during pregnancy and breastfeeding. Epidemiological data on the reproductive history of affected with MS men is not enough to conclude any relation between the disease and infertility. Moreover, there are not enough cohort studies to show the long-term effects of the used drugs on sperm quality. This report will focus on the interrelationship of reproductive health and multiple sclerosis in light of the most current scientific studies.

EFFECT OF MOZART'S MUSIC ON THE FETAL CIRCULATION

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The Pulsatility index (PI) is defined as the difference between the peak systolic flow and minimum diastolic flow velocity, divided by the mean velocity recorded throughout the cardiac cycle. It is a non-invasive method of assessing vascular resistance with the use of Doppler ultrasonography. The fetal Doppler PI measurement is well established in the monitoring of fetal wellbeing. The aim of our study was to identify the impact of Mozart's music on the resistance in fetal ductus venosus, umbilical artery, and middle cerebral artery. We have also evaluated the effect on the resistance in the maternal uterine arteries. This is a pilot study. Measurements were done on 60 healthy pregnant women. Our data showed significant rise in the PI in some of the fetal vessels. These results suggest that Mozart's music has an impact on the fetus, however we cannot define if the effect is positive or negative.

IN VITRO EFFECT OF TINCTURE OF *A. MUSCARIA* AND MUSCIMOL ON CONTRACTILITY OF CEREBRAL BLOOD VESSELS

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In recent decades, due to the progressive aging of the population, neurodegenerative diseases are among the most significant diseases, posing a substantial threat to the quality of human life. Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis are a group of neurodegenerative diseases characterized by diverse etiology, morphology and pathophysiological symptomatology, which ultimately lead to neuronal loss. Studies show that their development is also

caused by oxidative stress, accompanied by the formation and accumulation of free radicals. An interesting discovery in recent years is that the symbiosis occurring in some species of mycorrhizal fungi enhances the production of secondary metabolites, including bioactive compounds with potential antioxidant activity. In recent years, *Amanita muscaria*, known since ancient times as one of the most toxic and deadly mushrooms on earth, has attracted the attention of researchers for its potential antioxidant properties. Muscimol has been identified as one of the less toxic biologically active substances responsible for the psychogenic and sedative effects of *A. muscaria*, which is also a non-selective GABA-A receptor agonist whose levels are not affected by GABA transaminases (aminotransferases). It was found to have a ten times better affinity for the GABA-A receptor than GABA itself when administered in vitro. The aim of the present study was to evaluate in vitro the effects of *A. muscaria* tincture on the contractility of *a. basilaris*. Arterial segments with a length of 1.8 - 2 mm were mounted and tested on a dual- and multi-wire myograph (model 410A and 610M, JP Trading, Denmark).

Incubation of segments *a. basilaris* with *A. muscaria* tincture containing a concentration of 10 $\mu\text{mol/l}$ Muscimol, reduced vascular tone by 25%, compared to the control group. Incubating segments of *a. basilaris* with a solution of Muscimol, at a concentration of 10 $\mu\text{mol/l}$, lowers vascular tone by 30%, compared to the control group.

Individually applied, tincture of *A. muscaria* and mMuscimol, at a concentration of 10 μM , in vitro statistically significantly lower the vascular tone of rat basal artery.

ISOTRETINOIN – A SYSTEMATIC REVIEW OF ITS EFFECTS ON DERMATOLOGICAL DISEASES

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Acne is one of the most common and widespread skin conditions affecting the health as much as the quality of life of patients. A wide variety of acne treatments can be prescribed, both topical and systemic, depending on the severity. Isotretinoin or 13-cis-retinoic acid is an oral derivative of vitamin A that is used in the treatment of numerous dermatological diseases such as ichthyosis, hidradenitis suppurativa, rosacea, scarring alopecia, as well as in the

prevention of non-melanoma skin cancer, but it is mainly used in the pathology of the sebaceous glands. It is the first drug capable of inducing partial or complete remission of the disease. There are several studies in the literature describing its efficacy, also reporting side effects related to teratogenicity, effects on fertility, and occurrence of congenital malformations when used by pregnant women. The aim of the present review is related to the latest information on the characteristics of isotretinoin and comparing its efficacy and safety with other classical acne treatments, as well as with different treatment regimens, in order to find the dose with the best ratio between efficacy and safety.

PARKINSON'S DISEASE - SYSTEMATIC OVERVIEW ON NEW CLINICAL PATHWAYS FOR THERAPY

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Parkinson's disease is a progressive disease that affects the nervous system and the parts of the body controlled by the nerves. Symptoms start slowly. The first symptom may be a barely noticeable tremor of only one hand. Tremors are common, but the disorder can also cause stiffness or slowness of movement. Signs and symptoms of PD can include not only tremors but also muscle stiffness, impaired posture, and balance, loss of automatic movements, changes in speech, changes in writing, and slowed movement (bradykinesia). PD patients, who suffer from movement disorders, do not produce enough dopamine. They affect neurons in a part of the brain called the „Substance nigra”, which are responsible for producing dopamine. If more than 60 percent of the „Substance nigra” is affected, the well-known symptoms of parkinsonism occur. Treatment options for PD are limited, with most current approaches based on restoring dopaminergic tone in the striatum. However, they do not alter the course of the disease or treat the non-dopamine-dependent features of PD, such as freezing of gait, cognitive impairment, and other non-motor features of the disorder that often have the greatest impact on quality of life. As the understanding of the pathogenesis of PD increases, new therapeutic pathways are emerging. In this regard, the aim of the present review is to present the new therapeutic approaches that are close to or already in clinical trials for controlling the symptoms of PD.

EPILEPSY DURING PREGNANCY - A SYSTEMATIC REVIEW

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Epilepsy is the most common neurological disease in the world. This illness can affect any age group. Medico-social significance of the disease is large. This is due to the specific symptoms of epilepsy and the impossibility of predicting seizures. Epilepsy is a disease that does not have a direct effect on pregnancy. The most important step before conception is consultation with the doctor about antiepileptic drugs (AEDs) and how they can prevent the process. Spontaneous seizures pose a greater risk to the fetus than the correct AEDs dose. Consultation with a doctor is very important because it can prevent spontaneous seizures. When we talk about spontaneous seizures during pregnancy, they can have severe consequences for the fetus (slowing of the fetal heart rate, low birth weight, and preterm labor). The probability of seizures is different for each patient. Epileptic women can breastfeed unless they take AEDs, which can be life-threatening or can cause maldevelopment of the fetus. The probability of having a child with epilepsy is greater in couples in which the indicated is registered than in couple in which it is not. In this regard, the aim of the present systematic review is to summarize the last clinical advances in the control of the disease and the efficacy of applied therapies during and after pregnancy.

SYSTEMATIC REVIEW ON BENEFITS AND DISADVANTAGES OF PREDNISOLONE USAGE DURING GESTATION

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The evidence to date considering exposition to corticosteroids during pregnancy is limited and inconsistent. Corticosteroids are applied for their immunosuppressive and anti-inflammatory effects. They are used for the treatment of symptoms of autoimmune diseases since many standard immunosuppressive drugs, and biological agents are considered more precarious

during gestation or have potential unknown effects on the fetus. Synthetic corticosteroids are often used to manage the severity and flare-ups of patients' disease. These corticosteroids are developed to have increased glucocorticoid and reduced mineralcorticoid activity in comparison to the naturally found in the body cortisone and to have more powerful anti-inflammatory action. Although it is advised as optimal to take up to 20 mg a day of prednisolone during pregnancy, it is widely accepted that the dosage may be increased for treating more aggressive conditions. Uncontrolled autoimmune inflammation is considered more harmful to the mother and fetus than higher doses of the steroid. In this regard, the purpose of this review is to summarize the clinical trials connected to corticosteroids during pregnancy and possible unwanted consequences on the fetus, considering the positive effects these drugs have on treating different neurodegenerative diseases.

GUILLAIN-BARRÉ SYNDROME - AN OVERVIEW OF THE SYMPTOMS, TREATMENT AND PREDISPOSING FACTORS OF THE DISEASE

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Guillain- syndrome (GBS) is an inflammatory disease of the peripheral nervous system, identified with rapidly progressive, symmetrical on both sides of the body, arising to the upper body, muscle weakness, and in some cases, paralysis. Studies have shown the autoimmune nature of the disease. It is caused by damage of the peripheral nervous system caused by an attack of the myelin sheath of nerves. This can be caused by medical interventions and infections with a virus or bacteria. In the past two years, increased cases of GBS have been reported due to the Covid – 19 infection. After a progressive antibody-antigen reaction and demyelination of the nerve cells, the nerves lose their ability to receive and transmit signals efficiently. The result is an inability to feel heat, pain, and other sensations. GBS is difficult to diagnose in its early stages because of the common neurological symptoms. The most useful diagnosis are spinal tap, electromyography, and nerve conduction examination. All of them are based upon tests on the fluid from the spinal canal, the muscles and nerve signals. In spite of the fact that there is no exact cure for GBS, patients are given

two types of treatment that can speed up recovery - plasma exchange (plasmapheresis) and immunoglobulin therapy. The main idea of plasmapheresis is the release of plasma from certain antibodies, which make the immune system attack the peripheral nerves. In similar way, immunoglobulin therapy blocks the damaging antibodies that may contribute to Guillain-Barre syndrome with high doses of immunoglobulin. In recent years a new medicinal product for the treatment of GBS has appeared - eculizumab. Eculizumab is an antibody to the complementary protein C5 and can potently inhibit its activation. In relation to this, the aim of the present systematic review is to summarize the recent clinical achievements related to the control of GBS, the efficiency of the applied therapies, and the prerequisites for an increased percentage of diagnosed patients for the past two years.

THE EFFECT OF THE DEGREE OF DILUTION ON THE QUALITY OF EJACULATES OF THE SPECIES OVIS ARIES

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The reproductive performance of *Ovis aries* breeders is related to several characteristics, namely sperm quality, viability and fertilization ability, sperm quantity, sexual behavior, and the ability to serve ewes. Dilution of sperm helps maintain the viability and fertilizing capacity of sperm in vitro, while undiluted sperm deteriorates quickly after collection. In this regard, it is important to investigate the permissible degree of dilution of the sperm, and how it affects the quality of the ejaculate. This study aims to investigate the effect of the degree of dilution of seminal fluid on the motility, kinematic parameters, and viability of spermatozoa of the species *Ovis aries*. In the experiment, 10 ejaculates from breeders aged 3 to 5 years were examined during their insemination campaign. To accomplish this goal, all ejaculates were diluted 1:3, 1:6, 1:9, 1:12, and 1:24 with medium 6A containing sodium citrate, lactose, and sucrose. Sperm motility, kinematic parameters, and viability were analyzed by a computerized sperm analyzer (SCA, Microptic, Spain). BrightVit kit was used to assay viability. As a result of the conducted research, we found that the motility of spermatozoa significantly decreases at a dilution of 1:24 ($P \leq 0.05$). The same trend was found in the kinematic parameters (Rapid, VCL, STR, and AHL) of the spermatozoa with increasing dilution, however, the percentage of viable

sperm was not affected by the degree of dilution. In conclusion, the degree of dilution affects the motility and some of the kinetic parameters of spermatozoa of the species *Ovis aries*, but not their viability.

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EFFECT OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS ON SEMEN QUALITY AND MALE FERTILITY

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Selective serotonin-reuptake inhibitors (SSRI) are pharmaceutical drugs commonly used for a long-term treatment of depression, anxiety, and panic disorders in males of reproductive age. Another important use of SSRI (escitalopram/ fluoxetine/ sertraline) in men especially is the treatment of premature ejaculation (PE). According to a number of studies, antidepressant prescribing has increased by 35% in the last six years. On the other hand, even though the percentage of lifelong premature ejaculation is low in the general male population, SSRIs proved to be effective and of choice in treating lifelong PE. Studies have shown a negative effect of SSRIs on male-fertility hormones (testosterone, luteinizing hormone, and follicle-stimulating hormone). However, few studies have evaluated the potential impact of SSRI medications on sperm parameters such as DNA fragmentation, sperm motility, and sperm concentration. Thus, the aim of this review is to summarize a number of clinical studies done on the topic of semen quality in patients using this pharmaceutical drug and to evaluate the efficacy and adverse effects of SSRI on semen parameters as well as to assess the safety of this treatment among sexually active males who desire to have a child. Given the widespread and often long-term use of antidepressant medications, there is a clear need for further data regarding their impact on semen quality and male fertility.

STUDY OF THE MERCURY CONCENTRATION IN THE SEMINAL PLASMA OF MEN

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Mercury (Hg) is a heavy metal that occurs in the environment in minerals in the form of various inorganic and organic compounds. As a result of various human activities, it circulates freely in air, water, soil, and in ecosystems. There is a wide range of toxicological effects of Hg on humans. It attacks the central and peripheral nervous systems, the digestive, immune, cardiovascular and excretory systems. The most common routes of human exposure to mercury are seafood (when marine animals ingest Hg, it usually remains in their organism and builds up over time), burning solid fuels, pharmaceuticals, vaccines containing thimerosal, etc. In the last two years, there has been a discussion on the topic of increased Hg levels in humans after the administration of the Covid-19 vaccines and the consequences for the human organism. In this regard, the aim of our study is to determine the concentration of Hg in seminal plasma in unvaccinated and vaccinated men, as well as analysis of basic sperm parameters to establish sperm quality. To conduct the experiment, a total of 6 ejaculates of patients visiting the in vitro clinic were examined after signing informed consent. The patients underwent a sperm analysis, including general motility and morphology. The concentration of Hg was analyzed by the ICP-OES method. As a result of the conducted research, a higher content of Hg was found in the sperm plasma of the vaccinated men, while their motility and morphology were normal. From the studies carried out at this stage, we can assume that the high levels of Hg in the sperm plasma due to the administration of the vaccines for COVID-19 do not directly affect the main sperm parameters. For substantial/more specific conclusions on the topic, a larger number of patients and a bigger range of sperm analyses are needed to determine sperm fertility. Acknowledgment: The research was funded by the project "Investigation of the relationship between the concentration of metal ions and the oxidative status in human seminal plasma" at UCTM, Sofia – Bulgaria

SEASONAL VARIATIONS IN ENZYME ACTIVITY IN *OVIS ARIES* SEMINAL PLASMA

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Sheep (*Ovis aries*) are mainly seasonally polycyclic animals. Sperm production is influenced by many factors, including daylight length, climate temperature, humidity, and melatonin secretion, with females becoming fertile in early autumn and remaining fertile through mid-winter (October–February). Seminal plasma is a complex fluid that is a functional modulator of sperm function known to inhibit and stimulate sperm function and viability. Some seminal plasma proteins are capable of binding to the sperm plasma membrane and have an important role in sperm capacitation and egg fertilization. A variety of enzymes in the seminal plasma protect the sperm membrane from ROS-induced damage and lipid peroxidation through the activity of the antioxidant enzyme defense system. Regarding this, the aim of our study is to determine the biochemical changes that occur during the breeding campaign of the species *Ovis aries* and beyond. To fulfill the set goal, we have examined 6 ejaculates, spectrophotometrically. As a result, we found higher antioxidant enzyme protection outside the insemination campaign in glutathione (GSH) concentration and glutathione peroxidase (GPx) activity and non-significant differences in glutathione reductase (GR) activity. The enzyme activities of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were higher during the breeding season of the animals. In conclusion, a higher protective antioxidant system may help ensure adequate sperm fertilization potential when reproductive conditions are suboptimal. However, further research is needed to determine the mechanisms of action of the antioxidant enzyme and the relationships between indicators for determining sperm quality.

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INFLUENCE OF LEFLUNOMIDE IN LUPUS AND ITS SIDE EFFECTS ON THE REPRODUCTIVE SYSTEM

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Systemic lupus erythematosus (SLE) is an autoimmune disease often affecting multiple systems. Fertility has not currently been found to be affected in women with SLE, but disease-related factors, psychosocial effects of chronic illness, and drug exposure may impair gonadal function. In recent years, there has been a growing database on the efficacy of leflunomide in patients diagnosed with SLE. Its action is as an inhibitor of dihydroorotate dehydrogenase, it has an anti-proliferative and anti-inflammatory effect, which leads to a decrease in T-cells and B-cells. Leflunomide is a new immunosuppressive agent used in the treatment of rheumatoid arthritis as a disease-modifying antirheumatic drug. In various clinical studies related to the application, complete remission of the disease was reported. Unfortunately, studies have found that leflunomide can cause serious congenital disabilities. It is imperative that women who are planning to become pregnant, are breastfeeding, or are pregnant should not take this medicine. Both men and women should use contraception while on leflunomide. The drug can remain in the body for up to two years after the last dose. Women should not become pregnant, and men should not father children within two years of stopping leflunomide, unless the medicine is given to remove it from the body's system. Therefore, the aim of this systematic review is to summarize the new clinical data on the use of leflunomide, the adverse reactions it may cause in pregnant women, fetal abnormalities, adverse reactions in men, and its efficacy in SLE.

EVALUATION OF THE CONCENTRATION OF MACROELEMENTS IN SEMEN SAMPLES FROM MEN WITH REPRODUCTIVE PROBLEMS

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The declining male fertility is becoming a global problem. Recent retrospective evidence and major studies have shown relationships between reduced sperm quality and poor diet, increased levels of obesity, and exposure to environmental toxins. In this regard, the aim of this study is to evaluate the concentration of some macroelements in semen samples from men with reproductive problems in order to develop therapeutic approaches to protect and/or restore the quality of sperm. A total of 8 ejaculates were used for the experiments, divided into two groups according to male reproductive problems – normozoospermia and asthenozoospermia. The concentration of vital elements was studied by the ICP-OES method. As a result of the conducted experiments, lower concentrations of calcium (Ca) and magnesium (Mg) were found in the sperm plasma of men with reduced sperm motility (asthenozoospermia). No significant differences in the concentration of potassium (K) and sodium (Na) were observed between the studied groups. The small number of examined patients and the applied diagnostic methods do not allow us to draw definite conclusions, but to improve male fertility, we can suggest the intake of nutritional supplements containing Ca and Mg.

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EFFECTS OF MEDICATIONS ON MALE INFERTILITY

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Worldwide, 8%-12% of the population is experiencing problems with infertility, where 40%-50% of cases of infertility are caused by the male factor. Although the field of reproductive medicine is developing fast, the causes of male infertility are not fully clear. Factors: age, environment, social-economic and digestive status are a few of the preconditions for lowered quality of male gametes. The bond between virus infections and infertility is also researched for decades. Many viruses, including Zika virus, HIV, and cytomegalovirus, are found in the sperm, and according to different authors, they can have an impact on the fertility potential in men. Nowadays, it is not quite clear if the infection of these viruses or antiretroviral therapy have an impact on the spermal parameters. A number of studies describe the negative effect on spermatogenesis in laboratory animals after the use of ribavirin and interferon. In this regard, the goal of this report is to summarize the possibilities of unwanted reactions on fertility in both men and women, as well as the embryonic consequences after the use of different antiviral drugs.