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BOOK OF ABSTRACTS

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Contents

ORAL PRESENTATIONS

NEUROPLASTICITY IN ATTENTIVE BRAIN STATES MODULATES NEUROPHYSIOLOGIC MECHANISMS OF PAIN PERCEPTION IN HUMANS

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Background. Long-term contemplative practice has been demonstrated to induce neuroplastic changes in attentional networks for cognitive control (Yordanova et al., 2021). Notably, background attentive states can change the perception of pain intensity. In addition, previous research has revealed that the perception of pain intensity is substantially suppressed in long-term meditators who have extensive expertise in sustaining brain states requiring the activation of attentional networks (Nicolardi et al., 2022). It can be hypothesized that pain intensity suppression is associated with changes in the neurophysiologic mechanisms of pain perception due to the neuroplastic changes in background brain states in these long-term practitioners. However, no evidence has been provided so far for altered pain perception at the neurophysiologic level in experienced meditators.

Objective. To evaluate the oscillatory neurodynamics of pain-related brain potentials in experts with long-term contemplative practice.

Methods. EEG was recorded at 64 electrodes in 22 experienced meditators (MED) and 17 controls (CON) during nociceptive stimulation at the n. radialis of the left hand by means of a monophasic constant current during a rest condition. Pain event-related potentials (PERPs) were analyzed in the time-frequency domain (TF) after wavelet decomposition. They were spatially enhanced by means of current source density (CSD). The phase-locking value (PLF) was computed to analyse the temporal synchronization of oscillatory PERP components from different frequency bands. Functional connectivity during PERPs was evaluated using the phase-locking value (PLV), capturing the phase-synchronization between pairs of electrodes. The connectedness of contra-lateral (right-hemisphere) cortical areas with all other cortical regions (R-PLV) also was analysed.

Results. PERPs in both CON and MED were characterized by delta and theta-alpha components within 250 ms, and by beta, gamma 1, and gamma 2 TF components within 150 ms after nociceptive stimulation at motor and sensorimotor regions contra-lateral to the side of the pain stimulus. Importantly, the PLF, PLV, and R-PLV values of theta-alpha, beta, and gamma networks were significantly smaller in MED than CON at these regions.

Conclusion. These results provide original evidence for substantial differences between experienced meditators and controls during very early stages of nociceptive processing. These differences are suggested to be associated with the substantial dissimilarities in background alpha activity as indicating an altered attentional state. Because this study is in progress, it remains to be established how exactly PREPs are correlated with subjective scores of pain intensity, aversion, and identification, as well as with the EEG markers of background attentional modulation.

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EXECUTIVE CONTROL SYSTEMS AND CONTEMPLATIVE BRAIN STATES

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Background. The interest of modern society in empirical contemplative practices, as represented by meditation, is progressively growing. To understand neural mechanisms of meditation as a brain state, there is a need to put these practices on more solid scientific grounds and to conceptualize them from a neurocognitive perspective. Basing on extended empirical analyses, the first neurocognitive classification of meditation has been suggested (Cahn & Polich, 2006; Lutz et al., 2008). The major meditation categories are Focused Attention Meditation (FAM), where voluntary attention is focused on a chosen mental object in a sustained fashion; Open Monitoring Meditation (OMM), where attention is not explicitly object-focused, it rather involves the monitoring of the contents of experience and of mental processes in the present moment, and Loving Kindness Meditation (LKM) where awareness is focused upon a mental state of acceptance, wellbeing, and happiness. These neurocognitive definitions have guided the Executive Control Model according to which contemplative practice engages in the training of cognitive control systems in the brain, particularly processes of attention and cognitive monitoring.
Objective. To evaluate the functional involvement of attentional and cognitive monitoring processes during meditation.
Methods. High-density electroencephalographic (EEG) was recorded in a unique sample of highly experienced long-term practitioners (n = 22). Using the imaginary part of coherence, the EEG) synchronization of fronto-parietal (FP) and medial-frontal (MF) brain networks was analyzed in different frequency bands (theta, alpha, and beta) during different contemplative states in order to assess (1) whether the connectivity patterns of FP and MF networks are frequency-specific, and (2) if and how they are modulated by meditation style and expertise.
Results. Highly experienced meditators exhibited a strong theta synchronization of both FP and MF networks in left parietal regions in all mediation styles. The connectivity of intra-hemispheric theta FP attentional networks in the left hemisphere depended non-linearly on meditation expertise, whereas the connections between the left parietal and medial frontal regions supporting executive monitoring increased linearly with the progression of meditation expertise only during FAM. It also was found that inter-hemispheric FP connectivity in faster frequency bands (fast alpha and beta) increased linearly as a function of expertise.
Conclusions. These differential relationships with meditation expertise imply a more specialised role for the left parietal theta in processes of executive control. Inter-hemispheric fast-frequency connectivity is suggested to reflect enhanced awareness and facilitated access to consciousness.
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MODULATION OF EYE MOVEMENT CHARACTERISTICS BY PATTERN REGULARITIES

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Eye movements reflect the acquisition of information from the visual scenes and the underlying processes related to the spatial and temporal characteristics of the environment and the task demands. We studied the interplay between the structure of the visual stimulation and the gaze behavior in a task requiring the discrimination of patterns of different similarity. Ten observers were presented with pairs of images of the same number of square elements with varying groupings. The patterns contained 0, 1, or 2 matching chunks of elements. The changes in the fixation duration, saccade number, and saccade amplitude in the sequential blocks of trials were evaluated. The results show an increase in fixation duration and saccade number and a decrease in saccade amplitude. These results contradict existing data about the effect of learning of the image regularities on gaze behavior. The study's outcome is discussed in relation to the specifics of the task, the effect of chunks co-occurrence and repetition, and the processes involved in the visual selection of the distinguishing features for the discrimination performance.

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THE CASE OF MICROPLASTICS FROM THE PERSPECTIVE OF THE ONE HEALTH APPROACH: LITERATURE REVIEW

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The discovery of the pervasive presence of microplastics (MPs) in tissues and organs raises critical questions about their health effects on organisms, including humans. While studies on the impact of MPs have primarily focused on aquatic organisms, the potential consequences on humans remain a central scientific problem. Recent investigations into microplastics and nanoplastics (NPs) in mammalian models, predominantly mice, provide some understanding of the plastic exposure's uptake, translocation, and physiological repercussions of the. This review synthesizes findings from various studies, focusing on the tissue accumulation and effects of different types of MPs, including polystyrene (PS) and polyethylene (PE) with sizes ranging from 0.5 μm to 150 μm. Notable accumulations were observed in the gut, liver, and kidney, accompanied by a spectrum of toxicological responses. PS microspheres (5 μm and 20 μm) induced liver inflammation, altered lipid profiles, and compromised energy metabolism. Additionally, exposure to PS particles (0.5 and 50 μm) caused decreased body and liver weights, gut microbiota dysbiosis, and alterations in hepatic lipid profiles. The combination of PS and PE beads with organophosphorus flame retardants aggravated oxidative stress, neurotoxicity, and metabolic disorders. The severity of toxicity correlated with plastic size, concentration, and cellular/tissue uptake. Based on this review, can underscore the need for further research, especially in the context of human exposure, to comprehensively understand the health implications of microplastic exposure, bridging the current knowledge gaps and emphasizing the relevance of model organism studies in unraveling the complexities of microplastic toxicity.

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D-GALACTOSE AND ETHANOL-INDUCED MORPHOMETRIC CHANGES OF THE COLONIC MYENTERIC PLEXUS IN MOUSE MODELS OF AGING

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Aging of the gastrointestinal tract is related to various changes in the myenteric plexus. Aging models provide a fast, reliable and cheap method for studying senile-induced changes. D-galactose- and ethanol-induced accelerated aging models are widely used in age-related neurodegeneration because they produce bioactive aldehydes and reactive oxygen species associated with neurotoxicity and neuronal cell loss. This study aims to demonstrate the morphological changes of the myenteric plexus neurons in male ICR mice treated with D-galactose or ethanol and compare the two ageing techniques. Mice received D-galactose (3 mg/ml) and 1% ethanol with drinking water for six weeks at an average daily dose of 500 mg/kg bw. In both accelerated aging models, the neuronal size of myenteric neurons was significantly reduced along the entire length of the large intestine. However, no statistically significant differences in myenteric perikaryal sizes were observed between the two aging models. The mean area of the neuronal perikarya at the level of the proximal colon decreased by almost 30 %. Neuronal soma of the age-matched control group at the level of the distal colon had a mean value of 400 μm^2, and it was reduced by 40% in both aging models. Our results show that accelerated aging models cause a significant reduction in the sectional area of the neuronal perikarya and the ganglia at the level of the colonic myenteric plexus.

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IN VIVO STUDIES OF NEW INDOLE HYDRAZIDE–HYDRAZONE HYBRID AS MULTITARGET AGENT FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a complex neurodegenerative condition associated with a number of pathological processes such as chronic inflammation, increased oxidative stress and neuronal loss. Produced by the pineal gland in response to darkness, melatonin plays a crucial role in regulating circadian rhythms. The aim of the present study was to explore the effect of a newly synthesized melatonin analog with hydrazone fragment (**3c**) on behavioral and biochemical pathology in a rat model of melatonin deficiency and AD. A week before the icv amyloid-beta (Ab) or vehicle (veh) infusion, three groups of rats were operated on to remove the pineal gland. A matched (sham group) was exposed to the same procedure except for removing the gland. After pinealectomy (pin), the rats were divided into three groups based on their treatment with melatonin, **3c** and vehicle for 21 days. The Ab-pin rats treated with vehicle exhibited working memory dysfunction in the Y-maze test (Ist and IInd trail), object recognition test (ORT) and object location test (OLT) with concomitant elevation of the Ab level and tau-protein in the hippocampus compared to sham-veh group. The melatonin analog with hydrazone fragment showed protective effects similar to the positive control melatonin in the rat model of pinealectomy and AD. The **3c** compound corrected the Ab-induced memory impairment ($p > 0.05$ vs sham-veh group). It alleviated the pathogenesis associated with increased Ab and tau-protein expression in the hippocampus ($p < 0.05$ vs pin-Ab-veh group). The current data suggest that the novel melatonin analog with hydrazone fragment may be a novel agent for treating AD.

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THE ROLE OF NORADRENERGIC TRANSMISSION IN THE BASOLATERAL AMYGDALA FOR THE RETROACTIVE ENHANCEMENT OF INITIAL MEMORY CONSOLIDATION INDUCED BY PREDATOR ODOR EXPOSURE

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Remembering the neutral stimuli preceding salient events is essential for behavioral adaptability since they could be used as predictors for particular aversive or rewarding outcomes in the future.
Emotionally charged events elevate the release of noradrenaline (NA) in the basolateral amygdala (BLA). Multiple pharmacological studies have revealed that the infusion of synthetic NA or agonists of the β-adrenoceptors in BLA after learning - mimicking the physiological mechanisms of emotional arousal - enhances the consolidation of memories in the hippocampus in a retroactive fashion. We set out to test the role of BLA in the retrograde modulation of hippocampus-dependent memory by subjecting the animals to a mildly arousing emotional experience (modulatory event). We paired the neutral object-location (OL) task with subsequent exposure to a natural aversive stimulus to hypothetically induce greater levels of endogenous NA. Upon the completion of the sample phase of the task, groups of Wistar rats were exposed to coyote urine: a predator odor capable of inducing avoidance and other defensive behaviors. A subset of the experimental animals were infused with the non-selective beta-blocker propranolol in the BLA immediately following exposure to the predator odor. After 24h, animals underwent a test session to evaluate their performance in the OL task and were sacrificed 90 minutes later to extract the hippocampi. The collected samples were used to detect the levels of phosphorylated CREB (pCREB) and activity-regulated cytoskeleton-associated protein (Arc) - two molecular markers for experience-dependent changes in neuronal activity. Three different groups of animals were sacrificed 90 minutes after the exposure to the predator odor. This was done to evaluate if the presence of an aversive stimulus increases the levels of pCREB and Arc in the hippocampus during the consolidation of the memory for the OL task and if this memory-related activity could be interrupted by blocking the NA transmission in the BLA. Overall, the results we obtained indicate potential augmentation of the initial memory consolidation phase elicited by the aversive event. This is manifested by the higher levels of the aforementioned molecular markers in the animals that were not treated with propranolol after predatory odor exposure, suggesting that NA neurotransmission in the BLA during and shortly after the occurrence of emotional stimuli could influence neuronal plasticity in the hippocampus.
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SEX-SPECIFIC NEUROBIOLOGICAL CHANGES IN TWO PRENATAL MODELS

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Numerous experimental and clinical studies have shown that various prenatal influences can negatively affect intrauterine development and have lasting detrimental consequences on the offspring. Melatonin among the few hormones that cross the placental barrier without its molecule being changed, probably has an essential role in regulating the fetal circadian rhythm. The pineal gland of the fetus, which does not yet synthesize melatonin, is exposed to the mother's hormone. It is known that chronic exposure of adult rats to constant lighting disrupts the circadian rhythmic synthesis of a number of biochemical and behavioral indicators. Research on the impacts of progesterone indicates that beyond its role in reproduction, this hormone influences most of the body systems, including the immune, cardiovascular, excretory, respiratory, nervous, and male reproductive system. The aim of the present study was to investigate the emotional status of male and female adult offspring in two experimental prenatal rat models. In *Experiment*#1 one cohort of pregnant rats was exposed to constant light during the whole pregnancy (LL-veh group) while matched controls were set at standard 12/12 light (200 lux) regime (LD-veh group). Halve of the LL-exposed rats were prenatally injected by 20 mg/kg melatonin subcutaneously (sc) for 21 days. In *Experiment*#**2** experimental groups were prenatally treated with progesterone (10 mg/kg and 50 mg/kg) for ten days from G1 to G10 while the matched pregnant rats were injected with saline (C-veh group). Chronic prenatal exposure to the LL regime induced impaired emotional responses of male and female adult offspring, including decreased visits to the aversive central zone in the open field test, elevated plus maze test and reduced preference for sweet solutions. Prenatal melatonin treatment corrected behavioral deficits associated with emotional disturbance (anxiety and anhedonia). Prenatal treated with progesterone (PNP) male and female offspring demonstrated depressive-like responses with anhedonia in the sucrose preference test (SPT), increased immobility time in the forced swimming test (FST) and increased grooming time in the splash test compared to the matched controls ($p < 0.05$). Prenatal hormonal treatment in two doses also increased corticosterone levels in the hippocampus and plasma in both sexes. Based on the results obtained, we can conclude that prenatal treatment with melatonin is a beneficial tool for restoring the behavioral impairment of male and female offspring in a model of prenatal constant light exposure. Our results suggest that prenatal treatment with 10 and 50 mg/kg progesterone exerts a detrimental effect on emotional behavior in both sexes. Future studies must ascertain the underlying mechanism associated with these sustained behavioral abnormalities in adult offspring resulting from two different prenatal treatments.

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THE HAZARDS OF DEALING WITH RESPONSE TIME OUTLIERS

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There is a growing concern that some results in the field of experimental psychology are hard or impossible to reproduce. It has been suggested that one of the reasons for replication failures is that researchers explore various ways to process and analyze data and report only results which support the postulated hypotheses. In this work, we investigate how research integrity is affected by a particular source of methodological flexibility - the analysis of studies involving response time outliers.
A review of the published research on a single cognitive phenomenon, the Stroop effect, reveals there is considerable variability in how response time outliers are handled even in studies sharing similar designs and research questions. A series of computer simulations show that exploring multiple ways to handle outliers can result in the inflation of p-values and the distortion of confidence intervals and measures of effect size. Switching to Bayesian parameter estimations and probability distributions with heavier tails eliminates the need to deal with response times outliers, but at the expense of opening another source of flexibility. We end up by suggesting that the best way to counteract the publishing of false positive findings is by stimulating authors to include as many details about their studies as possible and by fostering critical attitude in reviewers and readers.

A CELL RESTING STATE AND INTERCELLULAR ENERGY TRANSFER AS A REFLECTION OF INTERACTION BETWEEN PUMPS AND ELECTROGENIC TRANSPORTERS

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The amount of energy that is transferred by pumps reflects the amount stored by ATP and it reflects the energy of the inner mitochondrial membrane that corresponds to the potentials of about -200mV – -180mV in normal conditions. In contrast to this, the surface membrane potential is about -90mV – -70mV for excitable cells. Significant energy losses would come if pumps would have to directly enforce cell resting state. Electrogenic transporters could provide a solution to that problem. A close and quantitative look at the problem confirms the conclusion. Open channels conduct 10 million charges/sec. or above. Na-Ca exchanger conducts about 5000 turns/sec while Na and Ca pumps work from one to hundred turns/sec (very occasionally a few hundred and only for Ca pumps). Thus, in normal conditions, a general problem for pump current deficit should emerge. A solution for that problem would be the formation of a massive network of parallelly working pumps whose efforts would be summarized by the help of electrogenic transporters. To obtain a mechanism for direct energy transfer between two cells one just needs that individual cells activate their endoplasmic reticulums at different moments of time. Once one cell's SERCA pumps are activated, that would drive the Na-Ca exchangers across membranes of both neighboring cells. The energy could be extracted from this process and transferred towards the K gradient with the help of inward rectifier K channels. Thus on the basis of resting state mechanism comes an energy transfer one.

E-POSTER PRESENTATIONS

SLEEP STATES AND THEIR DYNAMICS AS PROMOTERS OF SENSORIMOTOR AWARENESS

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Background. When people are exposed to environmental regularities, most of them remain unaware of the presence of such regularities. Yet, some people can gain implicit knowledge (ImK) about the regularities and just a few are able to consciously discover them. Acquiring such explicit knowledge (ExK) can induce a dramatic qualitative change in performance. It has been demonstrated that sleep is a critical factor for memory consolidation. It can induce stabilization, strengthening and reorganization of implicit and explicit memory representations formed before sleep. These processes can lead to knowledge awareness (ExK generation) and qualitative differences with pre-sleep performance. Two major questions are of relevance: (1) Does post-sleep ExK generation depend on pre-sleep ImK accumulation, and (2) Is there a sleep state that is responsible for ImK and ExK consolidation? Previous research employing tasks with hidden regularities has found that ExK generation after a whole night sleep does not depend on pre-existing ImK gain. However, when the roles of slow wave sleep (SWS) and rapid eye movement (REM) sleep have been separately targeted, SWS has been found to promote post-sleep ExK only in subjects with pre-sleep ImK, whereas ExK generation has been promoted by decreased REM in subjects without pre-sleep ImK.

Objective. To resolve this controversy, the present study tested a two-stage model of sleep function, according to which offline processing of information during sleep is sequential and requires multiple cycles of non-REM (NREM, including SWS, S2 and S1) and REM sleep stages. The hypothesis was that awareness after sleep might not depend on a single sleep stage. Rather, the interactions between different sleep stages (NREM and REM) might be critical for ExK generation.

Methods. To explore the role of overnight dynamic sleep macrostructure for ExK generation, the rate of sleep stage transitions was evaluated after implicit training of the Serial Reaction Time Task (SRTT), in which a determined sequence was inserted. Also, the frequency of transitions was assessed for a control night without pre-sleep training. After the experimental night preceded by SRTT learning, verbal recall was used to establish the amount of ExK. In the experimental and control nights, polysomnography was recorded. After sleep stage identification, the rate of sleep-stage transitions was analyzed and compared between participants who did or did not gain awareness of task regularity after sleep.

Results. Individual ability of ExK generation was strongly associated with increased rate of transitions between NREM and REM sleep stages, and between S1 and SWS. These effects were observed for both the experimental and control nights indicating a trait-dependent association between the rate of NREM–REM transitions and the amount of ExK after sleep.

Conclusions. Increased rate of transitions between sleep stages is an index of enhanced sleep lability. The results demonstrate that labile sleep promotes post-sleep knowledge awareness. Because this effect was observed for a night after learning but also for a night without pre-sleep learning, the association between sleep lability and knowledge awareness is an individual characteristics. Observations confirm the two-stage model and suggest that the interactions between sleep NREM and REM stages are involved in offline information processing, which promotes post-sleep awareness.

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BRAIN ACTIVATION PATTERNS DURING AUDITORY SELECTIVE ATTENTION: ERP GENDER DIFFERENCES IN ADOLESCENCE

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Background. Gender differences are found in neurocognitive processes of attention, memory, language, visual-spatial processing, etc. (Kimura, 1999; Vaquero et al., 2004; Neuhaus et al., 2009; Rubia et al., 2010; Kosmidou et al., 2015; Maciejewska et al., 2019). While gender effects have been observed in adults, developmental studies are scarce (Christakou et al., 2009; Hahn et al., 2010; Rubia et al., 2010, 2013).
Objective. The aim was to investigate the effects of gender on the neurophysiologic processes of selective attention in children and adolescents using event-related brain potentials (ERPs).
Method. An auditory selective attention task was used, in which two stimulus types with different probability (40% for targets and 60% for non-targets) were presented randomly to the right and left ear. Participants had to maintain lateralized internal attention either to right- or left-side stimuli in order to respond with the right hand to targets appearing on the attended side. Each condition comprised 4 stimulus types - target-attended, non-target-attended, target-non-attended, non-target-non-attended. Electroencephalogram (EEG) was recorded at 8 electrodes during task performance. Reaction times to targets and components of 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: In girls, the amplitudes of the early ERP N1P2 component was significantly greater at the frontal, central, and parietal regions of the left as compared to the right hemisphere. In boys, this lateralization was not expressed at frontal electrodes. In addition, the left-hemisphere frontal N1P2 dominance in girls was most pronounced for non-attended stimuli. Likewise, the amplitude of the early P2N2 in girls was significantly larger at the left versus right hemisphere. Accordingly, only at the left hemisphere was P2N2 significantly larger in girls as compared to boys. Late processing: The amplitude of the late parietal N2P3 component was larger in girls than boys for attended stimuli. These gender effects persisted throughout the age period under study (9-16 years).
Conclusions. The ERP analysis revealed that in 9-16-year-old children and adolescents, there are gender differences in the hemispheric asymmetries in the processing mechanisms of early selective attention: only girls demonstrate a prominent left hemispheric dominance mostly related to the early selection of non-attended stimuli. In the late stages of information processing, gender differences in parietal ERPs imply a greater activation in response to attended stimuli in girls than boys. The effects of gender on early and late mechanisms of selective attention may be due to gender-related differences in the functional maturation of brain regions engaged in selective attention and related gender differences in cognitive strategies.
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MENTAL STRESS IS ASSOCIATED WITH DIFFERENT PHASES OF AUTONOMIC NERVOUS SYSTEM REACTIVITY

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Background. Irrespective of their origin, environmental mental stressors are recognized as a high-risk factor for developing deviations in a variety of physiological, immune, and metabolic circuits. The continuous accumulation of stressful events appears particularly critical for dysregulation of the autonomic nervous system (ANS). Persistent deviations in autonomic balance can lead to serious somatic problems and diseases. It is well recognized that ANS functioning depends on the extent to which sympathetic (S) and parasympathetic (PS) components of ANS are simultaneously activated or inhibited.
Objective. The objective of this study was to explore the effects of environmental stress accumulation on the regulation of ANS balance.
Methodology. A total of 109 participants with high-risk professional occupations were enrolled. To assess life-stressor load in the preceding 24 months, the Holmes-Rahe stress scale was applied. To evaluate ANS functioning, an electrocardiogram (ECG) was recorded for 24 hours using a mobile easy-to-apply device. Based on 24-hour long records, heart rate variability (HRV) was analyzed in the time and frequency domains. Seven HRV indices were computed in the time domain, and low- and high-frequency HRV components (LF, HF) were computed in the frequency domain. The major assumptions were that high HRV values would reflect a stable S/PS balance, whereas HRV suppression would indicate a violated S/PS balance. Also, increased LF is proposed to reflect a dominating PS activation, and increased HF is proposed to correspond to a dominating S activation. To test the associations between life-stressors load and ANS functional state, regression analyses were used.
Results. Time-domain HRV parameters gradually increased and subsequently decreased with life-stressor accumulation in the preceding 24 months, indicating phases of compensation and later decompensation of ANS regulation. An increase in stress load was also associated with a progressive increase in S activity as captured by LF HRV component. The relationship between LF/HF ratio and Holmes-Rahe scores was, however non-linear, also pointing to an initial balanced activation of S and PS followed by PS hyper-activation and a subsequent deficit in PS engagement.
Conclusions. The present results confirm the associations between mental stress and ANS functioning. They demonstrate the presence of distinctive phases in ANS reactivity to progressive stress accumulation related to initial tolerance, compensation tension and subsequent decompensation of ANS balance upon especially high stress load. The results underscore the relevance of timely interventions for chronic stress reduction before a critical level of stress load is reached, such that a high-risk ANS imbalance can be prevented.
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GRANGER CAUSALITY IN THE FREQUENCY DOMAIN: APPLICATION TO ASSESS DIRECTIONAL CONNECTIVITY IN CONTEMPLATIVE BRAIN STATES

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Background. Neuroelectric cortical connectivity can be analyzed by employing a quantifier known as Granger Causality (GC). GC is a measure of directional connectivity as it reflects the direction of information flow. GC is based on the assumption that if the history of one time series X holds information about the future of a different time series Y, which is not present in the history of Y itself, then the X can be considered causal to Y. When applied to an electroencephalogram (EEG) recorded at different electrodes, the direction of information flow can be evaluated. Our previous work has demonstrated that long-term practice of contemplative brain states is associated with an increased flow of information from posterior to frontal cortical regions. Also, unidirectional functional connectivity has revealed that the connectivity of theta networks with a posterior integrating focus in the left hemisphere and the connectivity of alpha networks with a parieto-occipital integrating focus in the right hemisphere are enhanced in long-term practitioners (Yordanova et al., 2020, 2021). While these previous observations reveal important frequency-dependent changes in brain connections as a result of contemplative training, it remains unclarified how the direction of information flow depends on the frequency of involved brain networks.
Objective. To evaluate the frequency characteristics of networks that support directional connectivity in long-term contemplative practice.
Methods. EEG was recorded at 64 electrodes in 22 experienced meditators (MED) and 17 controls (CON) for 6 minutes 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. CG was computed in the frequency domain for 4 clusters combining electrodes in the left-frontal (C1), right-frontal (C2), left-posterior (C3), and right-posterior (C4) hemispheres. The Granger spectrum for each subject/condition/single trial/between-cluster pair was discretized into 1-Hz bins. GC values at each 1-Hz frequency bin were concatenated and subjected to a non-parametric pair-wise Kruskal-Wallis test, with subsequent false discovery rate (FDR) correction.
Results. GC representations in all states revealed a dominating broad peak with a maximum at 8-10 Hz. This peak was significantly larger in MED than in CON and was most prominent for directed transfer connections from posterior (C3 and C4) to anterior (C1 and C2) clusters. A similar smaller alpha peak was only observed in MED for inter-hemispheric information transfer between anterior clusters C1 and C2. Only in MED, this dominating peak was extended bi-directionally and encompassed frequencies from theta and beta bands. In contrast, these directed transfer connections in CON were supported by significantly larger delta activity.
Conclusion. The results demonstrate that long-term contemplative practice induces neuroplastic alterations in theta, alpha, and beta networks that specifically support directed information transfer from posterior to anterior regions and between anterior regions during the maintenance of different brain states.
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THE BENEFICIAL EFFECT OF THE GARDEN SNAIL HELIX ASPERSA EXTRACT ON INFLAMMATION IN AN EXPERIMENTAL RAT MODEL OF ALZHEIMER'S TYPE DEMENTIA

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Alzheimer's is the most common form of dementia, a general term for memory loss and other cognitive abilities severe enough to interfere with daily life. Contemporary hypothesis underlies the complex mechanism of Alzheimer's type dementia (ATD) which accounts for 60-80% of dementia cases worldwide. Snail extract (SE) obtained from garden snail Helix aspersa was analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Molecular masses of protein in mucus fraction >20 kDa was measured by an Autoflex™III, High-Performance MALDI-TOF. Obtained SE consists of 50% crude mucus extract and 50% fraction containing compounds with MW above 20 kDa. Rats were divided into 4 groups: Controls; Alzheimer's type dementia (ATD); ATD+SE; SE. The highest level of anti-inflammatory cytokine IL-10 was found in the group treated with snail extract. The level of IL-10 increased in the Alzheimer's dementia + snail extract group as compared to ATD. The lowest level of inflammatory cytokine TNF-alpha was detected in the control group and the one treated with snail extract. Most inflammatory cytokines were observed in the ATD group, whose concentration decreased significantly after administration of snail extract. Monocytes from only the snail extract group produced predominantly IL-10 without having high levels of TNF-alpha. Circulating CD43-positive monocyte decreased in the ATD group but increased significantly in the ATD+SE. Expression of CD11c integrin was normalized in the ATD+SE group, indicating probable traffic restoration and monocytes blood migration. In conclusion, our investigations demonstrated the anti-inflammatory properties of snail extract in Alzheimer's type dementia.

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EFFECTS OF NEWLY SYNTHESIZED PROLINE-CONTAINING TRIPEPTIDES ON ANGIOTENSIN I-INDUCED CONTRACTIONS OF ILEUM IN RATS

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Arterial hypertension is one of the most widespread socially significant diseases, which often triggers cardiovascular complications such as brain accidents (stroke) and myocardial infarction. As a consequence, it is the leading cause of premature death worldwide.

In most people with hypertension combinations of drugs are needed to effectively reduce blood pressure but polysubstance use can increase the risk of adverse drug reactions. One of the most recommended classes of antihypertensives are angiotensin-converting enzyme inhibitors (ACEIs), especially if the hypertension is accompanied by diabetes or heart and kidney failure, because of their good effect on these comorbidities.

Most currently used ACEIs are small molecules, short-chain peptides and their derivatives that have a hydrophobic, usually a proline residue at the C-terminal. Hydrophobic and branched amino acid side chains in this terminus are crucial for high-affinity binding of ACEIs to the two catalytic domains of somatic angiotensin-converting enzyme (ACE), with similar but not identical substrate specificities, responsible for the enzyme's activity and physiological function. Simultaneous and nonselective inhibition of both domains is associated with frequent side effects that often limit the use of the remedies. To reduce side effects, it is important to develop a new generation biologically active compounds as novel and highly potent ACEIs, selective more preferentially for one domain than another of the two domains of ACE.

The aim of the present study was to evaluate the ACE inhibitory potential of two newly synthesized proline-containing tripeptides - H-Leu-Arg-Pro-OH and H-Ile-Trp-Pro-OH, *in vitro,* on angiotensin I-induced (Ang I) contractions of the rats' ileum.

The target tripeptides were chemically synthesized by Fmoc–strategy of solid-phase peptide synthesis and characterized by nuclear magnetic resonance analysis.

The experiments were performed on isolated segments of the terminal ileum of rats. The freshly dissected (10-12 mm long) ileum preparations were individually placed in 4 ml water-jacketed organ baths, filled with Tyrode's solution, and- constantly aerated with carbogen 95% O_2/ 5% CO_2 at 37 ºC. Cumulative concentration-response curves to Ang I at increasing concentrations of 10^{-10} to 10^{-6}M were obtained in the absence or presence of the tested peptides, each applied 5 min before Ang I at two different concentrations - 10^{-6}M and 10^{-7}M. pIC_{50} and E_{max}% values were determined to evaluate the ACE inhibitory activity of the peptides.

The results showed that both of the investigated peptides affect the contractile responses of the ileal segments to Ang I in a similar manner to Lisinopril, a well-known ACEI, used as a reference drug.

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RELATIONSHIP BETWEEN PHYSICOCHEMICAL CHARACTERISTICS OF HUMAN BREAST MILK AND IDENTIFIED LACTIC ACID BACTERIA IN MOTHER'S BREAST MILK

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Breast milk is a highly dynamic and complex product containing nutrients, bioactive factors and microorganisms that change during lactation. All these components are of particular importance for the health of the newborn; they contribute to the maturation of his immunity, the development of the organs and the healthy microbial colonization of his gastrointestinal tract. Lactic acid bacteria occupy a central place in the probiotic microflora, both in- the mother's milk and the newborn's feces. Their amount and species diversity are influenced by many factors - diet and lifestyle of the mother, geographical location, macro- and microcomponents of breast milk, intake of antibiotics or probiotics, both by the mother and the newborn, mode and gestational week of birth, and genetic factors.

The aim of the study was to isolate and identify bacterial species from the feces of newborn children with potential belonging to lactic acid bacteria and search for a correlation with the macroelements in breast milk, established by physicochemical analysis. Samples from 10 mother/newborn tandem pairs and different lactation periods - 4 to 18 weeks were analyzed. Lactic acid bacteria was isolated by periodic enrichment and plating on MRS medium with additional supplements. The identification of the isolated microorganisms was performed by MALDI-TOF. The main macroelements in the breast milk - carbohydrates, lactose, proteins and fats were analyzed. Our results revealed a correlation between the mothers food diet and the mother's milk of composition . The most variable were carbohydrates from 5.87 - 10.67% while the most consistent macroelement was the proteins. The energy value of the breast milk was also investigated. The dominant group of the identified LAB were *L. rhamnosus, L. fermentum and L. paracasei*. We identified also *L. reuteri* and *L. oris*. It was observed that *L. fermentum* and *L. reuteri* develop at low levels of proteins and fats in the breast milk samples analyzed from us, but more studies are needed to confirm that the composition of the breast microbiota is in correlation with the tested physico-chemical characteristics.

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AGE-RELATED EMOTIONAL RESPONSES FOLLOWING PINEALECTOMY IN RATS

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The aging process influences anxiety levels in rats, and the pineal gland may play a role in modulating the inherent "life clock." This study aimed to investigate the impact of melatonin deficiency on specific behavioral and biochemical markers associated with accelerated aging. Lifespan, diurnal locomotion patterns, and anxiety responses were assessed in young adult, middle-aged, and old rats undergoing either sham surgery or pinealectomy (pin). Plasma corticosterone levels were measured before, 10 minutes, and 120 minutes after a stress-inducing procedure. Heat shock proteins (Hsp) 70 and Hsp 90 in the frontal cortex (FC) were evaluated using ELISA tests.

While melatonin deficiency did not affect lifespan or circadian motor activity, pinealectomy mitigated anxiety responses to novelty in young adult and old rats. Elevated corticosterone levels and a suppressed stress-induced release mechanism were observed in old pin rats compared to sham rats. Although there was a tendency for an increase in Hsp 70 in young adult pin rats, a decreased expression of Hsp 70 was noted in middle-aged pin rats compared to sham rats. Our findings suggest that the underlying mechanisms of age-related changes in emotional behavior in rats with pinealectomy vary between young adult and old rats, warranting further investigation.

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LONG-TERM ANTIHYPERALGESIC EFFECTS OF LOCALLY INJECTED HEMORPHIN IN A CARRAGEENAN-INDUCED RAT MODEL

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Hemorphins are bioactive peptides delivered by hemoglobin belonging to the atypical opioids with an affinity for opioid μ-, δ-, and k-receptors. One of the main disadvantages of opioid drugs is the development of tolerance after repeated administration. The aim of this study was to evaluate the anti-hyperalgesic potential of long-term treatment with natural hemorphin (Valorphin) in an experimental model of hyperalgesia in rats.

The experiments were executed on adult male Wistar rats. Paw edema was induced by intraplantar injection of 1 % λ-Carrageenan (CRG) administered twice (on the 1st and the 8th day). The peptide was injected intraplanar at a dose of 50 μg, 5 min before the CRG injection and then daily at the same dose 5 min before the pain measurement for 13 days. The mechanical pain threshold was measured daily using an analgesimeter Ugo Basile (Italy). The minimum pressure that induces a pain response such as withdrawal or struggle is defined as a pain threshold.

Our data showed that the hyperalgesia model induced by two injections of CRG produced a stable decrease in the pain threshold for 2 weeks. Valorphin significantly increased the pain threshold comparable to the reference drug Indomethacin injected at a dose of 800 μg intraplantar.

In conclusion, the obtained results reveal a significant and long-lasting antihyperalgesic effect of locally injected Valorphin without developing tolerance to the treatment.

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EFFECTS OF THE MICROPLASTICS ON THE GASTROINTESTINAL TRACT: LITERATURE REVIEW

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There is a growing interest in microplastics (MPs) – a modern-day polluter, not only because of their increment in the environment but also because of their not fully evaluated effects on human health. They are present in the atmosphere, soil, water, animals, especially in marine environments, and even in personal care products (e.g. toothpaste) and thus can become a threat to public health. This review is conceptualized to identify and summarise the effects of the MPs on the human gastrointestinal tract and microbiome. We considered reviews, systematic reviews, and meta-analyses from the last five years from the international database PubMed. Moreover, it is reported that MPs promote cadmium (Cd) uptake in plants and decrease shoot and root biomass. This fact leads to the need to evaluate the Cd concentration in plants and its accumulation in human organisms because of its cancerogenic effect. MPs are also found to alter the soil microbiome. In vitro and in vivo experiments with micro- and nanoplastics have been conducted and all of them concluded that MNPs have a negative effect on the experimental animals (mice, soil invertebrates and marine mammals) or cell lines. The toxicity of MNPs on the gastrointestinal tract leads to reduced intestinal mucus secretion, promotion of intestinal dysbiosis, depositions in the liver and intestines, and impairment in oxidative and inflammatory intestinal balance. MPs are linked to inflammatory bowel disease in humans. Therefore, it is necessary to explore the daily exposure to MNPs and its correlation with the microbiome as well as the signaling pathways in GIT. It is reasonable to hypothesize that a specific diet can have multiple adverse effects on the human digestive system.