The Dynamics of Central-Peripheral Stress Responses after Acute Psychosocial Stress: a Multimodal Perspective

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Tara Chand
Kohla/Indien

Tübingen
2022

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:	07.10.2022
Dekan:	Prof. Dr. Thilo Stehle
1. Berichterstatter/-in:	Prof. Dr. Martin Walter
2. Berichterstatter/-in:	Prof. Dr. Jennifer Svaldi

“The mind and body communicate constantly. What the mind thinks, perceives and experiences is sent from our brain to the rest of the body.”

- **Herbert Benson**

Summary

An acute stress response is a complex interaction of central and peripheral psychophysiological systems with unique temporal characteristics. Interestingly, the interaction represents a unique temporal characteristic. Investigating the dynamics of both brain and body signals during and after an encounter with a stressor allows us to understand the underlying principle of the acute stress response, which has been shown to be atypical in various psychiatric disorders. However, a detailed understanding of stress response is rarely investigated. Therefore, this thesis investigates two major approaches for understanding the acute stress response dynamics using simultaneous electroencephalography (EEG)-photoplethysmography-functional magnetic resonance imaging experiments in 39 subjects before and after the ScanStress task.

The EEG-derived vigilance indexes reveal a continuous decline at rest. Given the role of alertness in an efficient stress response, the effects of acute stress induction on EEG-derived vigilance metrics are of interest. Therefore, the first approach uses the dynamic analysis of psychophysiological stress responses after the acute psychosocial stress induction. The first study investigates the carry-over effect of acute psychosocial stress on vigilance and its modulation by the multicomponent over-the-counter drug neurexan, which has been shown to modulate the neuroendocrine stress response. By using dynamic analysis, six vigilance scores were calculated every two minutes before and after the stress induction during the resting state. The study revealed that stress delays the continuous decline of vigilance at rest. In addition, the stress-induced increase in mean vigilance levels at rest was correlated positively with the levels of perceived stress during the last month. In addition, the mean vigilance level exhibited a decrease after neurexan treatment compared to placebo intake.

Heart rate variability (HRV) can be viewed as an indicator of how well the adaptive regulation system in the brain reacts the peripheral environment. However, the relationship between the HRV and functional connectivity patterns in the brain networks in stressful situations is rarely investigated. Therefore, the second approach uses the multimodal approach to examine the interaction between different stress response systems. The study investigated the temporal association between HRV and

FC between the three core brain networks, namely the central executive network, salience network, and default mode network at baseline and after the psychosocial stress induction. In this study, the functional connectivity between three core brain networks and the HRV was examined by taking 60s window length. Furthermore, the temporal association between HRV and functional connectivity was investigated. A significant association was found between HRV and default mode network-central executive network functional connectivity at rest, which was significantly reduced after acute stress induction compared to baseline. These findings suggest that HRV co-fluctuates with the core brain networks selectively depending on the stress conditions.

In summary, acute psychological stress affects brain dynamics by exhibiting a delay in the continuously declining vigilance and keeping the brain in a more alert state even after the stressor disappears. Furthermore, the results suggest that EEG-derived vigilance metrics index not only stress-response but also the temporal dynamics of vigilance regulation. It can serve as a potential biomarker for the diagnosis and prognosis for stress-related disorders disrupting temporal characteristics of stress response dynamics and showing atypical stress response. In addition, the study revealed that stress affects the interactions among the core large-scale functional networks and physiological dynamics of the heart. The dynamic adaptation of the resources is crucial in a stressful situation; therefore, the stress alters the interaction between the brain and heart. The perturbation in this interaction may play an important role in developing and maintaining stress-related disorders. The thesis work provides novel insights and an understanding of the central and peripheral stress response dynamics, which show a huge potential for the diagnosis, prognosis, and therapeutic planning of individuals with neuropsychiatric disorders.

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Zusammenfassung

Eine akute Stressreaktion ist eine komplexe Interaktion von zentralen und peripheren psychophysiologischen Systemen mit einzigartigen zeitlichen Merkmalen. Interessanterweise stellt die Interaktion ein einzigartiges zeitliches Merkmal dar. Die Untersuchung der Dynamik von Gehirn- und Körpersignalen während und nach der Begegnung mit einem Stressor ermöglicht es uns, das zugrundeliegende Muster der der allgemeinen akuten Stressreaktion, und seine atypischen Abweichungen in verschiedenen psychiatrischen Störungen besser zu verstehen. Ein detailliertes Verständnis der Stressreaktion wird jedoch nur selten untersucht. Daher werden in dieser Arbeit zwei Hauptansätze zum Verständnis der Dynamik der akuten Stressreaktion untersucht, und zwar durch gleichzeitige Experimente mit Elektroenzephalographie (EEG), Photoplethysmographie und funktioneller Magnetresonanztomographie an 39 gesunden männlichen Probanden, vor und nach einer ScanStress-Aufgabe.

Die aus dem EEG abgeleiteten Vigilanzindizes zeigen eine kontinuierliche Abnahme in Ruhe. Angesichts der Rolle der Vigilanz bei einer effizienten Stressreaktion sind die Auswirkungen einer akuten Stressinduktion auf die EEG-abgeleiteten Vigilanz Metriken von Interesse. Daher wird im ersten Ansatz die dynamische Analyse der psychophysiologischen Stressreaktionen nach einer akuten psychosozialen Stressinduktion verwendet. Die erste Studie untersucht den ‘carry-over-effekt‘ von akutem psychosozialen Stress auf die Vigilanz und ihre Modulation durch das rezeptfrei erhältliche Multikomponenten-Medikament Neurexan, welches nachweislich die neuroendokrine Stressreaktion moduliert. Mithilfe einer dynamischen Analyse wurden alle zwei Minuten vor und nach der Stressinduktion im Ruhezustand sechs Vigilanzwerte berechnet. Die Studie ergab, dass Stress die kontinuierliche Abnahme der Vigilanz im Ruhezustand verzögert. Außerdem korrelierte der stressinduzierte Anstieg der mittleren Vigilanzwerte in Ruhe positiv mit dem Ausmaß des empfundenen Stresses im letzten Monat. Darüber hinaus sank die mittlere Vigilanz nach der Behandlung mit Neurexan im Vergleich zur Einnahme von Placebo.

Die Herzfrequenzvariabilität (HRV) kann als Indikator dafür angesehen werden, wie gut das adaptive Regulierungssystem des Gehirns auf die periphere Umgebung

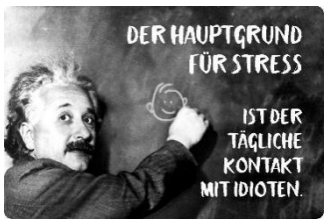
reagiert. Die Beziehung zwischen der HRV und den funktionellen Konnektivitätsmustern in den Gehirnnetzwerken in Stresssituationen ist jedoch nur selten untersucht worden. Daher wird im zweiten Ansatz der multimodale Ansatz verwendet, um die Interaktion zwischen verschiedenen Stressreaktionssystemen zu untersuchen. Die Studie untersuchte den zeitlichen Zusammenhang zwischen HRV und FC zwischen den drei zentralen Hirnnetzwerken, nämlich dem zentralen exekutiven Netzwerk, dem Salienznetzwerk und dem Default-Mode-Netzwerk zu Beginn und nach der psychosozialen Stressinduktion. In der Studie wurde die funktionelle Konnektivität zwischen den drei zentralen Hirnnetzwerken und der HRV untersucht. Außerdem wurde der zeitliche Zusammenhang zwischen HRV und funktioneller Konnektivität untersucht. Es wurde ein signifikanter Zusammenhang zwischen der HRV und der funktionellen Konnektivität des Standardmodusnetzwerks und des zentralen exekutiven Netzwerks in Ruhe festgestellt, der nach einer akuten Stressinduktion im Vergleich zum Ausgangswert signifikant reduziert war. Diese Ergebnisse deuten darauf hin, dass die HRV in Abhängigkeit von den Stressbedingungen selektiv mit den zentralen Hirnnetzwerken ko-fluktuiert.

Zusammenfassend lässt sich sagen, dass akuter psychologischer Stress die Dynamik des Gehirns beeinflusst, indem er die kontinuierlich abnehmende Vigilanz verzögert und das Gehirn auch nach dem Verschwinden des Stressors in einem wacheren Zustand hält. Darüber hinaus deuten die Ergebnisse darauf hin, dass EEG-abgeleitete Vigilanzmetriken nicht nur die Stressreaktion, sondern auch die zeitliche Dynamik der Vigilanzregulation indizieren. Sie kann als potenzieller Biomarker für die Diagnose und Prognose von stressbedingten Störungen dienen, die die zeitlichen Merkmale der Stressreaktionsdynamik stören und eine atypische Stressreaktion aufweisen.

Darüber hinaus ergab die Studie, dass Stress die Interaktionen zwischen den zentralen groß angelegten funktionellen Netzwerken und die physiologische Dynamik des Herzens beeinflusst. Die dynamische Anpassung der Ressourcen ist in einer Stresssituation von entscheidender Bedeutung; daher verändert der Stress die Interaktion zwischen Gehirn und Herz. Die Störung dieser Interaktion kann eine wichtige Rolle bei der Entstehung und Aufrechterhaltung stressbedingter Störungen spielen. Die Dissertation liefert neue Erkenntnisse und ein Verständnis der zentralen und peripheren Stressreaktionsdynamik, die ein enormes Potenzial für die Diagnose,

Prognose und Therapieplanung von Personen mit neuropsychiatrischen Störungen darstellen.

Synopsis



1. General Introduction

Stress is a consequence of the demanding lifestyles and competitive nature of our environment. Any type of challenge involving significant changes to a routine, including positive and negative life experiences, i.e., performance at the workplace, maintaining good relationships, or managing the financial situation, can be stressful. In modern society, stress has become a household word as well as an integral part of life. There is no doubt that acute stress is normal for all living organisms. However, chronic stress exposure can pose many problems that further affect social functioning (Bishop-Fitzpatrick et al., 2015). Moreover, prolonged stress exposure poses a significant impact on health status by increasing the risk of a variety of medical conditions such as hypertension, anxiety, insomnia, nervousness, restlessness, and depression (Shields & Slavich, 2017).

1.1. Stress: history and definition

The word stress comes from the Latin verb *strictus*, which means “to drawn tight.” Classically the term stress is used in mechanical engineering to define the deformation process of an object and describes various types of physical pressures. In the late nineteenth and early twentieth centuries, the concept of stress captured the attention of many disciplines, and researchers began exploring it through the narrow lenses of physiological processes or psychological phenomena. The seminal works of Claude Bernard (1813–1878), Walter B. Cannon (1871–1945), and Hans Selye (1907–1982), perhaps made the most significant foundational contribution to modern stress research.

Bernard observed that the body maintains a fixed internal environment despite of changing environmental conditions through physiological responses (Bernard, 1872). According to Bernard's concept of the internal environment "milieu intérieur", the bodily fluids are under the control of a central regulator. For example, to control internal temperature, the nervous system constricts and dilutes the blood vessels; and the liver regulates blood sugar through the glycogenic function. Consequently, these discoveries laid the foundation for homeostasis, which plays a critical role in stress research.

Further, Walter Cannon expanded Bernard's works and coined the word homeostasis - a process of maintaining a stable internal environment. He systematically explored the regulatory mechanisms and investigated the role of the sympatho-adrenomedulary (SAM) axis. According to Cannon, in the presence of stressors, the SAM axis activates, which changes heart rate and blood pressure and induces glucocorticoid release (Cannon, 1929; D. S. Goldstein, 2003). These changes in the physiological activations are subsequently exhibited in terms of the behavioral outcome of fight or flight responses, which are helpful to escape from the stressors or to avoid potential stressors.

Hans Selye, also known as the 'father of stress research', borrowed the term 'stress' from mechanical engineering and used it in biological systems to define the nonspecific response of the organism to a noxious stimulus (Selye, 1956). In medical school, he observed that patients often have similar complaints despite having different and distinct diseases. This group of common and nonspecific symptoms was referred to as the "syndrome of just being sick." A few years later, he observed that rats exhibited three non-specific symptoms after stress exposure to various stressors, i.e., enlargement of the adrenal glands, lymphatic atrophy, and peptic ulcers, and suggested that the effects are due to the unpleasantness (Selye, 1936). In subsequent experiments, he demonstrated that these nonspecific symptoms were due to the failure of adaptation to the environment (Selye, 1956; Viner, 1999). He later recognized that his findings coincided with the ideas of Walter Cannon on homeostasis, where the internal environment is maintained through the constant changes in bodily functions. He linked these nonspecific stress responses to the function of the hypothalamic-pituitary-adrenal (HPA) axis (Selye, 1979; Tan & Yip,

2018). He termed these adaptation failures as ‘general adaptation syndrome,’ also known as chronic stress. He distinguished acute and chronic stress responses and divided the total stress response into three phases: the alarming phase, the stage of resistance, and the exhaustion stage. The first two stages are described by acute responses to the stressor, whereas the last stage is chronic, which could lead to exhaustion from the stressor.

1.2. Types of stress

Individuals experience various types of stressors in both their personal and professional lives.

Based on the duration of stressors, the stress can generally be classified into two types:

1. Acute stress

It is the state of an immediate response to a single intermittent and time-limited exposure stressor. The adaptive responses elicited in response to an acute stressor usually re-establish the homeostasis balance. However, a signal exposure to stress induces a long-term functional glutamatergic release and dendritic atrophy structural consequences (Musazzi et al., 2017). Therefore, a dynamic dissection of acute stress suggests identifying key determinants of pro-adaptive versus maladaptive trajectories.

2. Chronic stress

It is caused by continuous exposure to the stressors for weeks, months, and years. Due to the chronic exposure of the stressors, the activation of the stress system often leads to a prolonged or repeated activation, which may adversely affect the mental and physical health of an individual. Chronic exposure to stress surpasses the physiological regulatory capacity, which further interrupt the reactivity of the HPA axis during stress (Stephens & Wand, 2012).

In addition to the duration, the types of stressors also play a key role in classifying stress. Based on the types of stressors, the stress can be further divided into two subtypes:

1. Psychological stress

It is due to psychological stressors such as anticipation or presence of negative emotions and demanding cognitive conditions. Experiences such as anxiety, frustration, anger, social threat, and trauma enable individuals to feel psychological stress. The psychological stress contains an aspect of social threat situations, such as social evaluation, social exclusion, and achievement also known as psychosocial stress. Maintaining the social self is the core psychological requirement of each individual. Any threat to the social self can lead to psychosocial stress, for instance, a negative judgment by others may result in psychological stress.

2. Physical stress

It is due to physical conditions such as injury, illness, and other unpleasant conditions such as high and low temperatures and mechanical pressures. These physical conditions associate with potential damage to body tissues (Peyron et al., 2000; Price, 2000; Tracey, 2005) and affect individuals' physical health conditions.

1.3. Stress response

Using mechanical stress as an analogy, when pressure is applied to an object, the object, in turn, places a force in the opposite direction to the applied force. If an applied force is higher than the object's reverse force, it deforms by losing its original shape; otherwise, it can regain its original shape. In a similar analogy, when a stressor is encountered in a biological system, it perturbates homeostasis, evoking chains of interrelated events in the organism to restore homeostasis (Monaghan & Spencer, 2014). These events are called stress responses, which employs the stress system, i.e., central/peripheral nervous system, and adrenal glands.

The stress responses depend on the environmental demand and help to reallocate the organism's resources to cope with the stress (Madsen et al., 1995; Peters et al., 2011). The stress responses involve efficient and evolutionary conserved complex systems at various levels in the central and peripheral nervous systems. These responses can be triggered not only by the real stressors but also during the anticipation of a stressful situation. This is very helpful to plan establishing a defence mechanism (Monat et al., 1972). In the presence of the real or anticipation of the stressors, the stress responses start immediately in the brain by evaluating the potential harmfulness of the stressors.

Following evaluation of the threat, two separate physiological stress responses are triggered in the body by activating, SAM and HPA axes. In response to the perception or anticipation of the stressor, the SAM axis is rapidly activated within a few seconds and triggers the release of catecholamines (epinephrine and norepinephrine [NE]) through the adrenal glands into the blood circulation and NE by sympathetic nerve fibers (Cannon, 1914; Joëls & Baram, 2009). In addition, a parallel release of NE occurs in the brain by the locus coeruleus (LC) (Ross & Van Bockstaele, 2021). The HPA axis activation is relatively slow compared to the SAM axis, which usually takes around 20-30 min until it shows the peak response after encountering a stressor. The activation of this axis initiates a series of neurochemical cascades that subsequently release cortisol in the blood, and reallocates the energy resources in brain and body (Peters et al., 2011). For instance, Madsen and colleagues, utilized the invasive Kety–Schmidt method to measure brain metabolic energy (global cerebral blood flow and global cerebral metabolic rates for glucose), demonstrated that the energy supply of the human brain increases by 12 % under mild stress in laboratory conditions (Madsen et al., 1995). These resource allocations help to deal with threatful situations, i.e., fight and flight response.

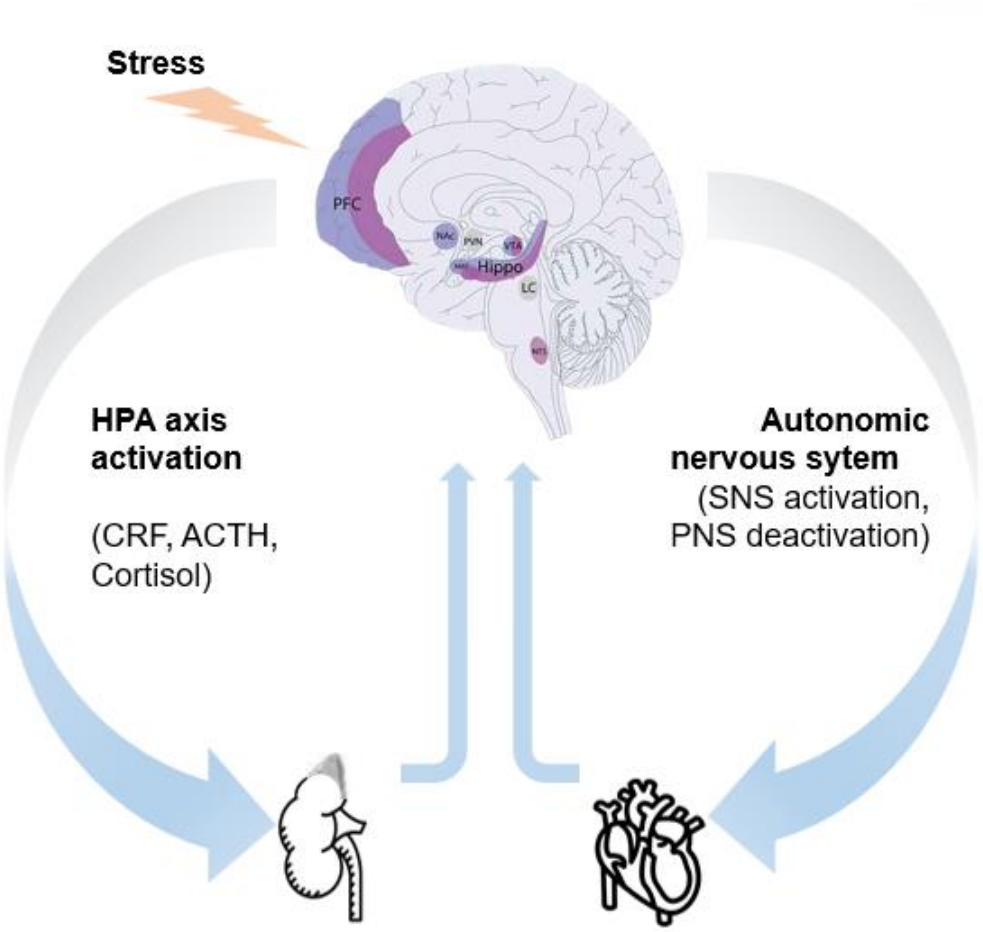


Figure 1: Central and peripheral stress response systems. The stress response system is activated in the brain and body by the central nervous system and peripheral nervous system (Figure adapted from Godoy et al., 2018; Wemm & Sinha, 2019)

1. Stress and CNS response

The first step of a stress response is to perceive the stressful situation. A stressor is perceived and transmitted by sensory pathways to different areas of the CNS, such as the thalamus and primary and secondary sensory cortexes. Previous research in humans and animals has described a set of additional neuronal circuits that play a vital role in the stress response, such as frontal association cortices, the hippocampal formation, and the amygdala (van Oort, et al., 2017.). The amygdala, a subcortical almond-shaped region, has anatomical projections and functional connections toward the brain areas related to the arousal level, thus enhancing the perception of the stimuli. The amygdala, along with other brain regions such as the dorsal anterior

cingulate cortex (dACC) and insula, forms the salience network (SN). SN is crucial to detect and filter the salient stimuli and recruit relevant functional networks that contribute to a variety of complex functions (Seeley, 2019). In response to a stressor, the amygdala activates the paraventricular nucleus of the hypothalamus (PVN), which is mediated by its extensive interactions with PVN-projecting regions, including the bed nucleus of the stria terminalis (BST) and brainstem structures such as nucleus tractus solitarius (NTS) and lateral parabrachial nucleus (Doewes et al., 2021; Godoy et al., 2018; Herman et al., 2003; Ulrich-Lai & Herman, 2009). The activation of the neurocircuitry in the PVN, where the corticotropin-releasing factor is synthesized, results in the release of adrenocorticotropic hormone (ACTH). ACTH subsequently releases cortisol from adrenal glands. Cortisol produces negative feedback by exerting its effects on the hypothalamus, pituitary, and hippocampus resulting in an inhibitory control on the HPA axis. Furthermore, the HPA axis activation is also influenced by NE and serotonin through projections from the LC and raphe nuclei, respectively. In parallel, PVN also sends information to the brain stem and activates the SAM axis (Nicolaides et al., 2015). The projections from PVN and other limbic areas, including the amygdala (Ordway, 2007), stimulate LC, resulting in NE release and activation of the SAM axis.

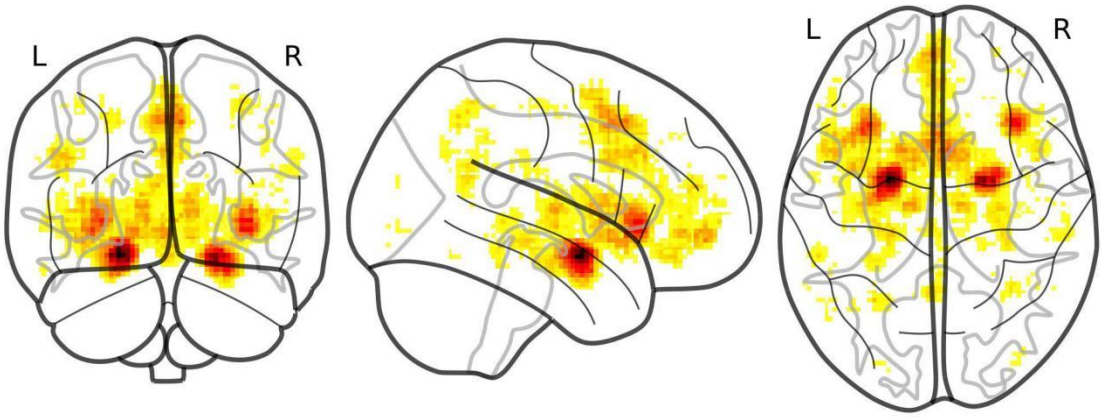


Figure 2: Neural correlates of stress. The stress atlas was derived from a term-based search for “stress” on Neurosynth (FDR-corrected p<0.05 /meta-analysis Yarkoni et al.; 2011) and was subsequently overlayed over the glass-brain atlas using the nilearn tool in Python

Furthermore, over the last decades, using brain imaging techniques, several brain regions and networks have been identified related to the stress response both during a variety of stress induction tasks as well as during rest (Godoy et al., 2018; van Oort, et al., 2017). During the rest, the intrinsic brain functional networks facilitate stress response by processing the stimuli. The communications within and between networks are crucial for stress processing, and any perturbations in these communications result in various neuropsychiatric disorders. Menon and Uddin proposed a triple network model. which emphasizes that three canonical networks SN, default mode network (DMN), and central executive network (CEN), are disrupted across various psychiatric disorders. These disruptions play essential roles in various functions, i.e., self-referential processing, the saliency of the stimuli, and executive control functioning (Menon & Uddin, 2010). Notably, these three brain functional networks are also influenced by stress induction. The stress response can be characterized by the perturbation of the within or between network interactions (Menon, 2011; Soares et al., 2013; van Oort et al., 2017a). Moreover, stress induction leads to various changes in neural dynamics reflected in terms of dynamic network interactions as well as behavioral and cognitive adaptations due to dynamic interactions between the SMA and the HPA axis (van Oort et al., 2017a).

2. Stress and peripheral response

The bodily signals result from the adjustment of various systems, i.e., cardiovascular, skeletomuscular, neuroendocrine, and autonomic nervous systems (ANS). The releases of both axes, SAM and HPA, systemically promote energy mobilization and metabolic changes throughout the body (Peters et al., 2011). After perceiving potential stressors, the body encourages specific physiological activities helpful for emergency situations and halts the other long-term projects in the body, i.e., digestion and reproduction. Consequently, glucose pours out from the storage sites. Furthermore, the heart rate and blood pressure also increase to accelerate the transport rate of the nutrient and oxygen to the critical muscles. The activation of the SAM affects several physiological mechanisms and enables fight and flight responses, i.e., dilation of pupils, the elevation of blood pressure, constriction of blood vessels, inhibition of salivary gland activities. In contrast, the HPA axis activation results in the ACTH release, which stimulates the adrenal glands to synthesize and secrete cortisol

hormone into the bloodstream. The cortisol helps perform metabolically demanding actions by activating glucose production.

1.4. Stress response dynamics

Aforementioned organisms exhibit a shift in the distribution of resources to perform optimum actions to cope with stress. This shift occurs over a period of time and demonstrates unique temporal properties. The stress response shows multidimensional properties during the first hour followed by stressful events, by illustrating the temporal trajectory of activation patterns of the SMA and HPA axis (Musazzi et al., 2017). As shown in figure 3, during the stress response, SMA is activated immediately after an encounter with a stressor and deactivated rapidly. In contrast, HPA shows a gradual response and reaches its peak response around 15-30 minutes, even when the stressor is removed (Van Den Bos et al., 2013).

The stressors can trigger a dynamic shift in brain network activation, allowing the organisms to reorganize their neural resources to meet cognitive demands (Hermans et al., 2014). For example, Young and colleagues showed dynamic changes in the network cohesion as a function of arousal level in the reallocation of neural resources strengthened SN activation during acute stress (Young et al., 2017). The finding suggests that rapid and vigilant reactions are crucial for adaptive responses in response to threats.

Furthermore, the temporal trajectory of stress responses can also be understood in terms of the different phases of the stress including in the presence of the stressors called the stress response phase and after the stressor called the recovery phase or carry-over phase. Due to the continuous nature of the stress response, it is impossible to make a sharp distinction between both phases (van Oort et al., 2017a). In the presence of stressors, homeostasis is disrupted, and the stress responses are activated (Hermans et al., 2014; Schulz & Vögele, 2015; Wager et al., 2009). During the post-stress recovery period, allostatic processes facilitate a return to homeostasis (Karatsoreos & McEwen, 2011). Hence, directly after inducing stress, there is still a state of the continued stress response, from which we can recover more with time; therefore, a carry-over effect of stress can be observed. For example, increased

activation and connectivity have been observed in the SN during the stress periods (Dedovic et al., 2014; Seo & Sinha, 2011; Sinha et al., 2016) as well as shortly after the stress task (van Marle et al., 2010a). The increased SN activity leads to an optimization of threat detection by inducing a hypervigilant state (Young et al., 2017). On the other hand, a decreased activation in CEN has been reported during and after the stress, which can be helpful in reducing the further resource allocation to the CEN. Interestingly, corticosteroids normalize higher-order cognitive functions (Dolcos et al., 2014; Henckens et al., 2009; Hermans et al., 2014).

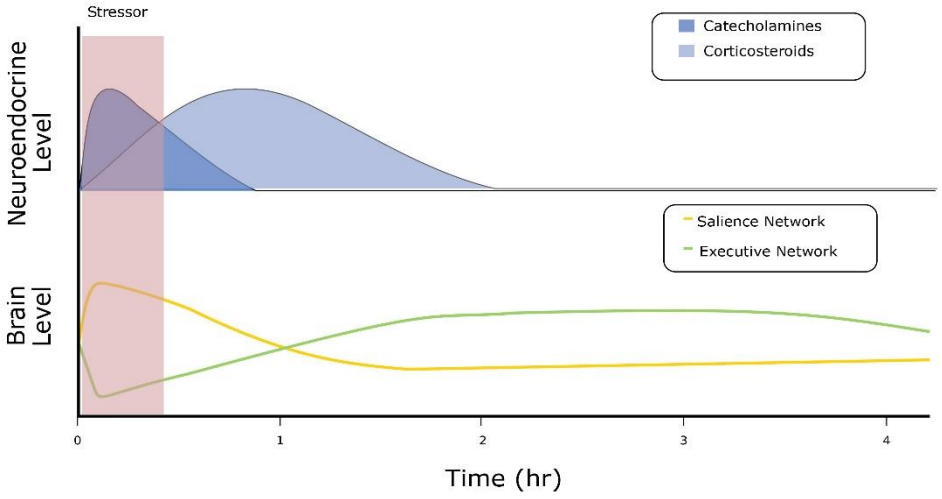


Figure 3: The stress response dynamics. The figure shows the temporal trajectory of stress response as a function of time from the brain level (bottom) and the neuroendocrine level (top). (Adapted from Hermans et al., 2014, Trends Neurosci).

1.5. Acute stress induction

For research purposes, acute stress can be induced experimentally using physical and psychological stressors. An ideal stressor must demonstrate changes in (ideally more than one) endpoints such as cortisol blood level or heart rate indexing a stress reaction. The most commonly used psychosocial stressors involve either a high-order cognitive task such as mental arithmetic or a combination with social evaluative situations. Furthermore, the cold pressure and electric shock test, in which participants

must endure an uncomfortable physical stimulation, the types of physical stress, are also used to induce acute stress.

The stress response can also be induced within the fMRI scanner, which has enabled the examination of neural circuits associated with the stress response non-invasively. In recent years, various paradigms, i.e., Montreal Imaging Stress Task (MIST), ScanSTRESS, Social-Evaluative Threat (SET), aversive video paradigm etc., have been designed which elicit different cognitive and emotional coping processes (Dedovic et al., 2005; Dickerson et al., 2008; Henckens et al., 2009; Streit et al., 2014). For example, social evaluative stress and time pressure are combined with a cognitive load in MIST and ScanSTRESS tasks to increase the cognitive load. In contrast, the SET task requires anticipation of the social evaluation without a cognitive challenge (Dedovic et al., 2005; Dickerson & Kemeny, 2004; Streit et al., 2014). In the aversive video paradigm, the highly aversive video material induces psychosocial stress passively (Henckens et al., 2009).

ScanSTRESS paradigm

This thesis used an adapted version of the ScanStress paradigm to induce acute psychosocial stress (see Figure 4). As a first-time application of the ScanStress task in a cross-over design, we demonstrated a successful stress induction (Herrmann et al.; 2022). In this task, alternating stress and control 40-s blocks were presented to the participants. In stress blocks, participants were asked to perform two cognitively demanding tasks (serial subtraction and mental rotation) under time pressure. At the same time, a live video of the jury panel of the experimenters in white coats was presented to the participants to induce a social-evaluation threat. The jury mimicked a disappointing face while participants showed poor performance. In addition, negative feedback related to the speed and correctness of the responses, such as "Work faster!" or "Error!" were also presented directly on the screen. In control blocks, the stress factors such as the social-evaluative and cognitive demands are largely relaxed, including simple figure and number matching activities with no time constraints. The judging panel does not react to the participant's performance in the control blocks, thereby minimizing the social input. The rotation task preceded the arithmetic task, and there were no pauses between the two consecutive events/scans.

Figure 4: ScanSTRESS Paradigm. The figure shows two different tasks A). mental rotation and B) subtraction task) of ScanSTRESS paradigm in the performance phase.

1.6. Monitoring stress response

Following the stress induction, many parameters, including subjective and objective measures, are used to quantify the stress response. Even though there is no gold standard measure of the stress response, the available measures enable investigating the specific properties of the stress response. While the subjective measures focus on the qualitative descriptions of participants' conscious feelings of stressors, the objective measures include the stress response's conscious and unconscious feelings.

1. Subjective measures of the stress response

Several self-report measures have been developed to measure the subjective experience of stress to quantify acute stress responses. The most common and straightforward subjective approach to quantifying stress is using the Visual Analog Scale (VAS) (Lesage et al., 2012; Smeets et al., 2012). The participants were asked to rate their stress level within a range (e.g., 0 = "not at all" and 100 = "extremely"). Further, the VAS can also be used to assess several emotional states. A stressor can be perceived as stressful based on several emotional states assessed on VAS, such

as stress perception, anxiety, calmness, and nervousness (Smets et al., 2019). The VAS has the advantage of being a quick way of assessing perceived stress and avoiding misunderstandings during questionnaire-based assessments due to communication. It also offers the advantage of capturing momentary stress at multiple points. The photographic effect/stress meter (PAM/PSM) also measures an individual's subjective stress experience repeatedly over time (Dorsey et al., 2020; Haim et al., 2015). In MPSM, a mobile-modified version of PSM, the participants were asked to select an image that best captured their stress levels. Anxiety-related assessments, such as the State-Trait Anxiety Inventory (STAI), were commonly used to evaluate an acute stress response following a stressful event (Frazier & Parker, 2019; Shahidi et al., 2013).

2. Assessment of the peripheral stress response

The use of objective measures of the stress response has increased due to the advancement of technologies. In contrast to the subjective measures, it allows researchers to evaluate the stress response of the subjects who are unable to express their feeling of stress (Hufnagel et al., 2017). The peripheral stress responses can be indexed by monitoring the activity of the endocrine, cardiovascular, and sympathetic nervous systems. The stress hormones, i.e., cortisol and catecholamines, can be monitored in various body fluids or excreted. The catecholamines can be sampled from urine, plasma, or saliva (Cohen et al., 1997; Dimsdale & Moss, 1980). Since the ANS innervates salivary glands, activation of the sympathetic system also affects their secretion. Therefore, salivary alpha-amylase has been used to quantify the catecholaminergic reactivity that is easily accessible and obtained (Nater & Rohleder, 2009; Sapolsky et al., 2000). The cortisol assessment can be performed using plasma, salivary, urine, or hair sample; however, different sample collections are used to measure different types of stress (Dorsey et al., 2020; Gormally & Romero, 2020). The hair cortisol is used as an objective measure to quantify the degree of chronic stress and subserve as a biomarker for chronic stress (Lee et al., 2015; E. Russell et al., 2012). The acute stress responses are reliably captured through the salivary, serum, or plasma samples (Gormally & Romero, 2020). Although cortisol is considered the best reliable biomarker for stress, the peak cortisol response can get approximately 20 to 30 min after stressor onset (Kirschbaum et al., 1993).

During stress, heart rate (HR) and blood pressure usually increase, and heart rate variability (HRV) decreases due to the increase/decrease in sympathetic/parasympathetic activities (Castaldo et al., 2015; Kim et al., 2018). The HRV can be calculated in a time and frequency domain (Shaffer & Ginsberg, 2017). The standard deviation of normal-to-normal interval (SDNN), root mean square of successive differences between normal heartbeats (RMSSD), and NN50 are the standard time-domain analysis measures to assess the stress and found to be decreased in stressful conditions (Kim et al., 2018; Shaffer & Ginsberg, 2017). In the frequency domain, a power spectral density analysis provides information about power distribution as a function of frequency. HRV spectral components such as high-frequency band (HF) and low-frequency band (LF) are commonly used for frequency domain analysis. The stress-induced changes are characterized by a decrease in HF and an increase in LF (Kim et al., 2018).

3. Assessment of central stress response

Stress response in the central nervous system can be monitored non-invasively with the help of neuroimaging techniques such as functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and electroencephalography (EEG). Each imaging modality has its unique set of features concerning the signal sources (spatial or temporal resolutions). For example, fMRI provides a high spatial resolution to study the neuro-circuitry of the stress response with a lack of temporal resolution. By contrast, EEG provides a high temporal resolution but a low spatial resolution.

Functional magnetic resonance imaging

The fMRI is a promising tool for understanding stress-associated brain regions (de Kloet, 2003; De Kloet, 2004; Veer et al., 2011). The fMRI can be acquired with or without an explicit task condition within an MRI scanner. In task-based fMRI, the relative changes in the BOLD activation from baseline are measured during the subject performs a stressful task. In contrast, the stress-related perturbation can be measured just after the stress task during the resting period without an explicit task. Generally, two analytic approaches, namely functional segregation and functional integration, are used to investigate the stress-induced perturbation in neural activity using fMRI. Functional segregation is concerned with the local function of specific brain regions.

In contrast, functional integration concerns the functional relationships or connectivity between different systems within the brain (Lv et al., 2018). In task fMRI, relative changes of the bold activations are the main focus to investigate. Several studies have shown that psychosocial stress activates brain regions involved in cognition and affection. For example, recently, a meta-analysis conducted by Qiu and colleagues showed that several brain areas, including the insula, thalamus, middle cingulate cortex, and amygdala, were consistently activated in acute stress. (Qiu et al., 2022). The resting state observes the spatiotemporal pattern of the brain's intrinsic activity. Several studies have shown stress-induced changes during the resting state both within and between the functional connectivity in three canonical brain networks, SN, DMN, and CEN (Vaisvaser et al., 2013; van Oort et al., 2017b). The FC of the amygdala was shown to be perturbed by stress and during the regulation of the stress response (Maron-Katz et al., 2016; van Marle et al., 2010b). Using aversive movies to induce acute stress, Van Marle and colleagues found an increased FC of the amygdala to the anterior insula (AI) and dACC after stress compared to the control condition. van Oort et al., 2017 reviewed stress-induced changes in functional brain networks in healthy individuals and found a consistent increased activity and connectivity in SN and DMN. Most stress paradigms do not induce changes in the CEN during and after the stress task (van Oort et al., 2017b). However, increased activation in CEN was found during acute stress if the stress paradigm included a higher-order cognitive element (Koric et al., 2011; Lederbogen et al., 2011).

Electroencephalography

Acute stress-induced changes have been reported in EEG signals (Alonso et al., 2015; Sanei & Chambers, 2007; Sulaiman et al., 2009). In an acute stress condition, a person is characterized by higher arousal and tonic alertness to maintain attention towards the external environment and fast responsiveness. EEG is a promising tool for investigating arousal-related brain state changes (Olbrich et al., 2012). The stress response can be indicated in terms of the spectral characteristics of the brain waves based on the EEG frequency powers. For example, an increase in beta power has been shown during acute stress and is constantly used to index anxiety, excitement, and tension (Alonso et al., 2015; Sanei & Chambers, 2007; Sulaiman et al., 2009). The increase in alpha power has been shown in the subjects who experience chronic

stress (Peng et al., 2013). The frequency components of EEG signals, i.e., delta, theta, alpha, beta, and gamma, are associated with the various affective states (Reuderink et al., 2013). For example, Theta (4–8 Hz) and Gamma (>30 Hz) are associated with positive effects. The posterior delta (0.5–4 Hz) and global alpha (8–13 Hz) activities are associated with increased arousal levels. To measure the cortical arousal level, Hegerl et al. developed a computer-based algorithm (Vigilance algorithm Leipzig, VIGALL). This algorithm classifies EEG segments into different EEG-vigilance stages based on EEG activities' frequency and topographical distribution (A1, A2, A3, B1, B2/3).

In summary, subjective and objective measures monitor the stress response, provide unique perspectives, and index a specific aspect of the stress system. To comprehensively understand the stress system, measuring every aspect of the stress is crucial. The different stress response systems can be congruent (one measure overlaps with other measures) or incongruent (one measure does not overlap with other measures). The incongruence could be the consequence of the interaction in the different stress response systems. The HPA and SNS systems interact and compensate each other during the stress to achieve the optimum response. One system influences the other and results in certain psychological states. As the HPA is relatively slow; therefore, the baseline HPA axis values (ACTH & cortisol levels) play an important role in the subjective experience of the stress. For example, on the one hand, higher HPA axis reactivity and moderate SNS responsivity resulted in a sensitive pattern for the stress response showing higher emotional stability and inhibitory control. On the other hand, high HPA and SNA responsivity resulted in a vigilant pattern for the stress response, showing high trait anxiety and increased attention to threat. Monitoring multiple systems in the same study has been recommended to understand the basic principles of stress regulation better.

1.7. Multimodal data acquisition

In recent years, due to better access to hybrid devices and the advancement of multi-model methods, researchers can simultaneously assess the distinct aspects of the stress response. Different modalities are more sensitive to one dimension than the

other in a stress response system. Therefore, the complementary information can be measured using multimodal imaging, which allows cross-validate the distinct sources of the results and provides advantages of the cross-information. The combination of structural and functional MRI can, for instance, provide a structural aspect of brain function. With multimodal imaging and mapping of the stress response on both the central and peripheral level, together with measuring the behavioral aspect, we can investigate the neural correlates of the behavior and develop a theoretical model to map stress-related brain regions and behavior.

In multimodal studies, the data can be acquired simultaneously (EEG/fMRI, fMRI/ECG, EEG/ECG) or separately (PET and structural, diffusion, or functional MRI). The data can be acquired from both the central and peripheral nervous systems or only one of them. During simultaneous data acquisition, the same states of the subjects are measured with different modalities; therefore, stronger intra-individual correlations are exhibited between different modalities, whereas, in separate conditions, subjects will be in different temporal states and are more sensitive to the variables that are relatively stable over time. Each method has its advantages and limitations. Combining the data can complement the limitation and provide rich information.

EEG-fMRI

EEG provides excellent temporal resolution but has a low spatial resolution. On the other hand, fMRI provides good spatial resolution but low temporal resolution. Complaining EEG-fMRI acquisition helps to investigate the spatio-temporal neural basis affective system and provides the 'when' and 'where' pieces of information of the affective signal processing in the brain (Ritter & Villringer, 2006). The integration of the EEG-fMRI data can be performed using various approaches, which can be symmetrical or asymmetrical integration approaches. In asymmetrical integration, information from one modality is used to predict the information of the other modalities. For example, fMRI data can guide or constrain EEG source reconstruction.

ECG/PPG-fMRI

The Combination of ECG/PPG-fMRI helps to investigate the functional and functional association of the heart and brain. The fMRI provides the advantages of non-invasively

measuring the neurovascular interactions within the brain. The ECG and PPG signals are acquired to capture the cardiac activities inside the scanners. The advancement of the recent technique makes it possible to simultaneously acquire both the signals and enhance the progress of examining the mechanism of CNS and PNS together. The use of the ECG and PPG trade off between accuracy and set-up time. The ECG measures the heart's electrical activity and provides a reliable measure of the cardiac cycle. However, the magnetic field considerably influences the signal. The PPG detects the blood volume changes during the cardiac cycle, and this signal's waveform is associated with the systolic and diastolic phases of the cardiac cycle. The contact probe is placed on the fingertip or earlobe to acquire the PPG signal. In the MR environment, the signal is acquired from the fingertip; therefore, the signal is less affected by the MR gradient due to its distance from the magnetic field. Due to the blood circulation to the fingertip, the PPG signal has a limitation in temporal resolution. It can only estimate cardiac activity (Schumann et al., 2021).

1.8. Therapeutic intervention for stress relieving

Stress can be overcome with various medications, including benzodiazepines and serotonin reuptake inhibitors. However, long-term use of these medications can have adverse effects (Rosenberg, 2006). Previously, the Neurexan® (Nx4) over-the-counter (OTC) in Germany showed to be a useful agent for lower stress-related symptoms such as insomnia and restlessness (Doering et al., 2016; Hubner et al., 2009). Nx4 has been shown to change stress reactivity in rats and demonstrates the same EEG pattern as antidepressants and anticonvulsant drugs (Dimpfel et al., 2012).

2. Own work related to the central-peripheral stress responses

2.1. Research aims and expected outcomes

The stress response is a multidimensional dynamic response. Therefore, a dynamic analysis of the temporal trajectory of the stress response in conjunction with using multimodalities is necessary to understand the underlying principle of the stress response comprehensively.

This work aims to examine the dynamics of stress-induced central and peripheral response, which is summarized in two papers.

The first paper (Chand et al., 2021) investigated the effect of acute psychosocial stress induction on the temporal patterns of vigilance state, which was indexed by EEG. Six discrete vigilance states were calculated for every two minutes over a 12-minute resting state. A hyper-vigilance regulation after the stress induction and a reduction in vigilance in subjects treated with Nx4 compared to placebo were expected.

In the second paper (Chand et al., 2020) investigated the temporal association between HRV (bodily signal) and functional connectivity (FC, brain signal) between the three core brain networks, namely CEN, SN, and DMN, at rest and immediately following the psychosocial stress induction. A flexible, functional association of HRV with FC between the core brain networks before and after acute stress induction was expected.

The major contents of the two papers are summarized below after describing their study designs.

2.2. Study design

The current dissertation's research findings are based on the same study sample of a clinical trial conducted as a randomized, placebo-controlled, double-blind, two-period cross-over design. A group of 40 mildly to moderately stressed healthy male volunteers aged 31–59 years were recruited. Since males and females have different

stress responses that are influenced by hormonal differences produced in part by the menstrual cycle (J. M. Goldstein et al., 2010; Saladin et al., 2015), only male subjects were recruited for this investigation to limit stress response variability. One subject was excluded due to an incidental finding; therefore, 39 subjects were employed for simultaneous EEG, fMRI, and PPG acquisitions. In total, three 12-min resting-state measurements were performed, including baseline (RS0), pre (RS1), and post (RS2) stress resting state. After the baseline (RS0) session, participants received a single dose of Nx4 or placebo, followed by the pre and post-stress resting state scans. Psychosocial stress was induced by using the ScanStress task.

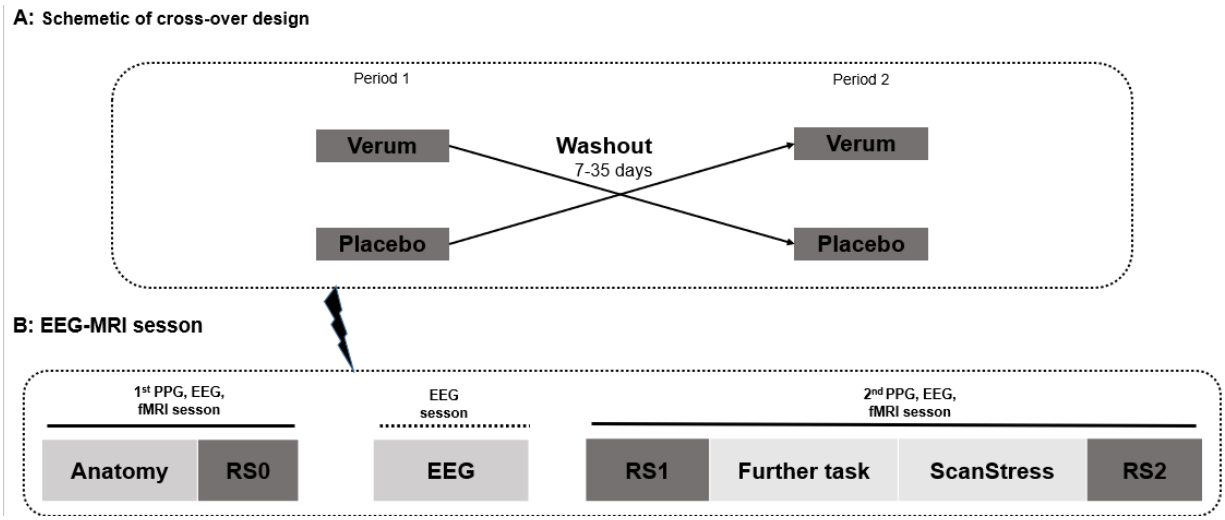


Figure 5: The schematic overview of the randomized, placebo-controlled, double-blind, two-period cross-over study design. (A) In period-1, half of the subjects were treated with verum and half with placebo. The treatment order was cross overed on period-2 (B). In each period, the fMRI scans were acquired in two sessions. During each fMRI session, simultaneous PPG and EEG data were also acquired. The ScanStress paradigm was performed in the second session, which contains two resting-state measurements before and after the tasks.

The pre- and post-stress task design allowed us to examine the impact of stress on the resting state, while multimodal and continuous data acquisition allowed us to explore the dynamic nature of stress by using different measures of the stress response on central and peripheral levels. At the central nervous system level, EEG signal was used to calculate vigilance scores, and functional connectivity between

core brain networks was calculated by BOLD signal. On the peripheral level, PPG data were used to calculate HRV measures. In the first study, we used vigilance score to investigate the carry-over effects of stress and the potential role of Nx4 in mitigating the effects. In the second study, we investigated the relationship between the brain and body signals using BOLD and PPG signals.

2.3. EEG revealed improved vigilance regulation after stress exposure under Nx4

Background/Hypotheses

Vigilance refers to being aware, attentive, and characterized by sustained attention. The hypervigilance state is part of a neuroendocrine stress response, which triggers a flight-or-flight response. Nx4 has been shown to modulate neuroendocrine stress responses. In this study, we hypothesized that stress would induce a hypervigilance state, which could be normalized by the intake of NX4.

Data and Methods

EEG data from three resting-state sessions: at baseline (RS0), pre- (RS1), and post-stress task (RS2) were used to calculate vigilance state for both Nx4 and placebo groups. Using the VIGALL 2.1 algorithm, each 2-s segment of the EEG epochs was classified into one vigilance state. In total, seven vigilance states along a wake-sleep axis were calculated.

Results and Discussion

As shown in figure 6, subjects exhibit a hyper-vigilance state after the stress induction (RS2), which continuously declines over the period of resting state before the stress. The stress-induced changes in mean vigilance (RS2-RS1) are positively correlated with PSS-10 scores in the placebo group ($R = 0.46$, $p = 0.02$). Further, subjects treated with the Nx4 compared to placebo had significantly lower mean vigilance levels and spent substantially more time in the lower vigilance state B2/3.

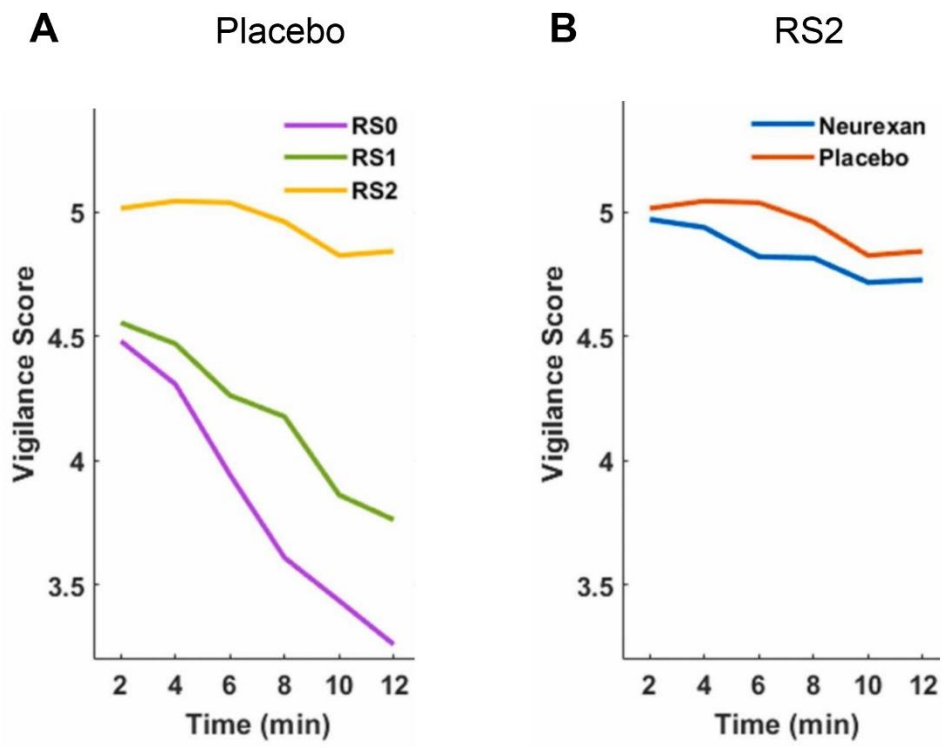


Figure 6: Vigilance fluctuations. Figure showing the time course of EEG-vigilance fluctuations under placebo for three resting-state sessions (A) and both placebo and Nx4 for only RS2 (B).

These findings indicate that vigilance levels remain high even after the stressor disappears, which might serve to stay aware of surroundings in stressful situations. Moreover, the dynamic analysis of the vigilance levels showed that vigilance levels continuously declined in pre-stress conditions and after placebo intake (Figure 6). This continuous decline in vigilance at rest is in line with the previous literature showing subjects exhibit unstable wakefulness in the scanner and drift into sleep within three minutes of typical resting-state experiments (Tagliazucchi & Laufs, 2014). Following the acute stress induction, not only the mean vigilance scores increase, but also the vigilance level stayed at a high level during the scanning. The increase in the vigilance after stress suggests that the EEG-derived vigilance metrics might be an indicator of the brain mechanism of the vigilance regulation as an amount of subjective experience of the stress from the past month as measured by the PSS-10.

A change in vigilance levels under Nx4 may be linked to its effect on the amygdala, which is known to be critically involved in stress responses through altering vigilance (Klumpp & Amir, 2009). Indeed, an increased amygdala activity in stress-related disorders was also shown during the rest (Drevets, 2003; Etkin & Wager, 2007). Interestingly, Herrmann et al., 2020 recently reported a decreased activity in the centromedial amygdala (CeMA) in response to negative stimuli (emotional face matching, Hariri task) in the Nx4 arm compared to the placebo arm (Herrmann et al., 2020). The CeMA projects to cholinergic neurons in the whole cortex of the brain, which modulates vigilance (Davis & Whalen, 2001; Fadok et al., 2018). Moreover, the CeMA also plays an important role in maintaining sustained attention on potential threats, modulating the vigilance level (Cain et al., 2002; Torrisi et al., 2018).

Conclusion

In conclusion, the ScanStress task reliably induces a hyper-vigilance state in the post-stress resting state. After stress induction, the mean vigilance scores were increased. The stress-induced increase in vigilance level is positively correlated with perceived stress from last month as measured by PSS-10, suggesting that the EEG-derived vigilance metrics might be an indicator of the brain mechanism of the vigilance regulation as an amount of subjective experience of the stress from the past month. Additionally, the expected decline in the temporal trajectory of dynamic vigilance scores at rest was not seen after stress induction. Nx4 improves vigilance regulation by reducing the mean vigilance score in post-stress resting state conditions.

2.4. Heart rate variability as an index of differential brain dynamics at rest and after acute stress induction

Background/Hypotheses

Traditionally the heart has received much attention in terms of the interaction not only between the body and the brain but also in terms of the ecological relations of the human organism to its environment. The interaction between the heart and the brain has been given a central role in the psychophysiological system and was proposed to be flexible and dynamic in order to facilitate a coordinated response to ever-changing conditions. This system, as it was described by Thayer and colleagues in 2012 in the

frame of the neurovisceral integration (NVI) model, functions as a “super-system” through which the operations in multiple domains of the brain functioning such as perceptual, motor, or interoceptive are integrated into likely adaptive responses (Thayer et al., 2012). Recently, Smith and colleagues (2017) extended the NVI model and put forward that three core brain networks, namely DMN, SN, and CEN, regulate the interaction between the body, the brain, and the environment (Smith et al., 2017). They proposed that the HRV can be used to index the dynamic interaction between brain networks and the heart in case of a salient event such as an encounter with a stressor (Smith et al., 2017; Thayer & Lane, 2000). Therefore, we hypothesized that the minute-by-minute association between HRV and FC between the three network pairs (DMN-SN, DMN-CEN, and SN-CEN) would differ at rest and after acute psychosocial stress induction.

Data and Methods

The same study was used as in research focus 1, but only the placebo arm was included. Using a sliding window approach (60-sec window size and 50% overlap), we calculated dynamic FC (dFC) between SN-CEN, SN-DMN, and DMN-CEN. RMSSD (root mean square of succeeding interbeat intervals) was chosen as the HRV metric and was calculated for each window. The multilevel mixed effect model was used to examine the effect of stress on the minute-by-minute association between HRV and FC because the common summary-statistics model cannot take the variance of first-level data (sliding windows).

Results and Discussion

The results of research focus 2 showed a differential role of HRV on temporal dynamics of the interaction between the three canonical brain networks, namely DMN, SN, and CEN, by examining their relationship across 60s-time windows and at two different resting-state scan sessions in baseline and just after a Scan-Stress Task. A significant association was found between RMSSD and DMN-CEN dFC at rest, which was stronger than the association between RMSSD and SN-CEN dFC. Moreover, dFC between DMN and CEN showed a weaker correlation with RMSSD after the stress task compared to baseline. These findings extend previous neuroimaging literature not only by examining the dynamic co-fluctuations of HRV and FC between intrinsic

networks at rest but also by testing the carry-over effect of stress on the interplay between HRV and interaction of intrinsic brain networks.

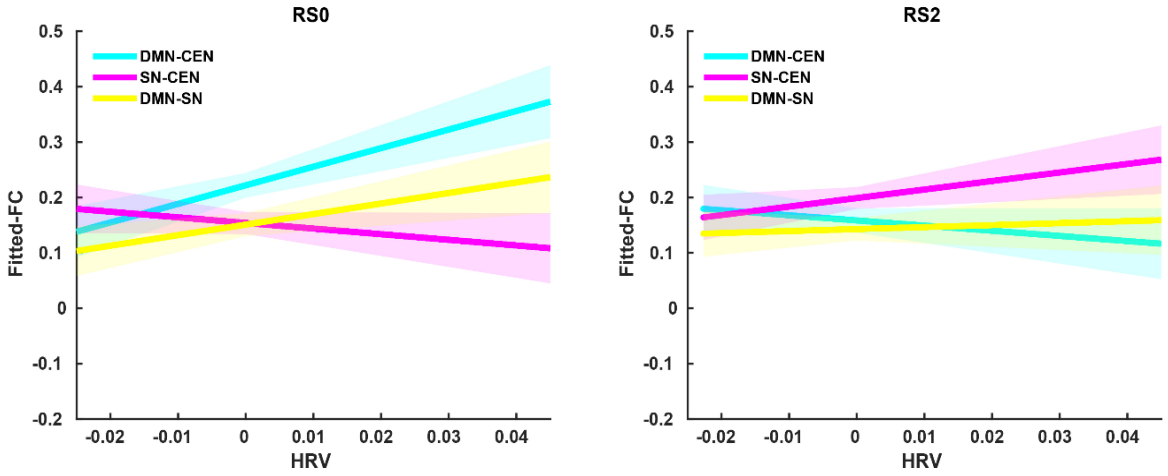


Figure 7: The association of heart rate variability (HRV) and functional connectivity (FC) between network-pairs in pre- and post-resting state sessions. The multilevel linear mixed effect model showed a significant correlation between HRV and FC between DMN-SN, and DMN-CEN during baseline (RS0) which was significantly reduced after acute stress (RS2) in comparison to baseline session. Shaded areas indicate standard error.

The crosstalk between intrinsic brain networks flexibly varies depending on the cognitive demand and affective feature of the task (Dixon et al., 2017, 2018; Fan et al., 2016; Liang et al., 2016). Moreover, the interplay between the heart and the brain was hypothesized to vary depending on the current and expected needs of the organism (Smith et al., 2017). This flexibility in the coupling within the psychophysiological system is believed to underlie the adaptive behavior by allowing the brain to undergo continuous transitions among various functional states due to the slowly fluctuating cognitive, emotional, and especially autonomic functions (Young et al., 2017). Based on the NVI, heart rate variability (HRV) can serve as an index of the interaction between the heart and brain networks (Smith et al., 2017; Thayer & Lane, 2000). Considering that acute stress alters the FC patterns between core brain networks (Hermans et al., 2014; Quaedflieg et al., 2015; Vaisvaser et al., 2013; van Marle et al., 2009; van Oort et al., 2017b) and decreases HRV (Castaldo et al., 2015; Kim et al., 2018) , we expected an altered association of HRV to dFC between network

pairs and HRV after acute stress induction. Indeed, the findings of this study showed that the interaction between brain networks and the heart is responding to acute stress induction in a flexible manner. Specifically, RMSSD showed a weaker correlation to dFC between DMN and CEN following acute stress. This is in line with the fact that the DMN activity is dominant at rest (Buckner et al., 2008; Greicius et al., 2003), and this dominance disappears during a task (Raichle et al., 2001) or after stress (Quaedflieg et al., 2015; Vaisvaser et al., 2013; van Marle et al., 2010b; van Oort et al., 2017b). Moreover, it also supports the extended NVI model, where the CEN and DMN were proposed to take roles respectively in vagal control and representation of the conceptual significance of the overall situation by integrating somatic and visceral information with the self-concept (Smith et al., 2017).

Conclusion

In conclusion, the brain network interaction metric is a predictive measure for HRV. Furthermore, we investigated how this metric is related to HRV after we completed the stress task. The results indicate that HRV may contribute to dynamic interactions between brain networks.

3. General Discussion

The present work investigates the brain and the body stress response dynamics and their interactions. This study investigates the dynamic and complex process of stress responses using a multimodal data acquisition approach and dynamic data analysis. These approaches are crucial to understanding the spatiotemporal properties of the stress response comprehensively.

The first study (Chand et al., 2021) examined the carry-over effects of acute psychosocial stress on the temporal dynamics of the EEG-derived vigilance metrics. The study revealed that acute stress delayed the expected vigilance decline in the resting state. Additionally, the stress-induced changes in vigilance correlated with the perceived chronic stress measured by PSS-10. This suggests that the EEG-derived vigilance metrics might be a useful indicator of the brain mechanism of vigilance regulation in response to acute stress, which is likely affected by perceived levels of chronic stress. The second study (Chand et al., 2020) investigated the effect of acute psychosocial stress on the temporal association of the brain and body. The association between dFC of three canonical brain networks - DMN, SN, and CEN - from the brain and HRV from the body were examined. A significant association was found between HRV and DMN-CEN FC at the baseline resting state. Moreover, this association was significantly decreased after acute stress induction compared to the baseline resting state, suggesting that the interaction between the brain networks and the heart depends on the psychological state.

The relation between vigilance and cardiac activity

The present work revealed increased cardiac activity (Chand et al., 2020) and mean vigilance levels (Chand et al., 2021) after the acute stress induction. The stress induction triggers a series of endocrine changes by activating the SAM and HPA axis and elicits a specific pattern of physiological responses such as an increase in heart rate and elevation in vigilance level. In particular, the SAM axis secretes adrenaline and NE into the blood circulation, and the HPA axis helps in secreting NE in LC via CRF (G. Russell & Lightman, 2019; Stephens & Wand, 2012). The secretion of the NE modulates sleep-wake state transitions (Morgane & Stern, 1975) and contributes to an elevated level of arousal. Furthermore, the activation of the SAM axis also

contributes to the changes in the autonomic regulation of psychological response. Subsequently, the PNS withdraws its inhibitory control over SNS, resulting in fight and flight responses. In addition, the decreased parasympathetic activity leads to cardio-acceleration, resulting in an increase in HR and a decrease in HRV. Furthermore, the elevated level of vigilance, increase in HR, and reduction in HRV are normalized when the stressor disappears. However, this normalization takes place gradually, and the carry-over effect of stress shows itself in HR, HRV, and vigilance levels after the stressful situation ends. In agreement with the above-stated mechanism, both studies showed increased cardiac activity and mean vigilance levels after the acute stress induction. These findings are in lines with previous studies implicating vigilance stages association with cardiac activities (Olbrich et al., 2011; Ehrhart et al., 2000, Kubota et al., 2001).

Vigilance and heart-brain interaction

The changes in the vigilance state have been shown to be associated with the changes in cardiac and global neural activity, which might mediate heart and brain interactions (de Munck et al., 2008, Olbrich et al., 2009). In particular, converging evidence demonstrates changes in HRV or vigilance or arousal attributing to the fluctuations in the FC (Chang et al., 2013a, 2016; Chang et al., 2013b, Haimovici et al., 2017; Thompson et al., 2013; Wanget al., 2016, Young et al., 2017). Furthermore the baroreceptors are responsive to HRV with the change in pressure on the vessel wall, and the activation of baroreceptors induces a slow-wave activity/ lower vigilance stage (Bonvallet et al., 1994). The relationship between the vigilance state and the heart-brain interaction can be explained by the Neurovisceral Integration (NVI) model. The NVI proposes that vagal control is regulated by hierarchically organized brain networks, which account for the integration of cognitive, affective, and peripheral physiological information. The brain regions in the hierarchically lower level mainly integrate current interoceptive information from the body. The higher levels integrate exteroceptive or past information to regulate the energy expenditure for present and future needs. The interplay between the heart and the brain could vary depending on the organism’s current and expected needs and the level of arousal (Chang et al., 2013; Smith et al., 2017; Young et al., 2017). Notability, three canonical networks investigated here, namely DMN, SN, and CEN, were placed at the highest levels of

the hierarchy. The synchronous activity of these networks may provide a “global workspace” that allows the emergence of conscious representations that are significant to the overall state of the organism, which in turn control orientation functions, including vigilance level (Dehaene, 2014; Barrett, 2016; Smith et al., 2017). At the top of the hierarchy, the CEN selectively amplifies/maintains internal representations in a top-down manner (Barrett and Satpute, 2013). Under resting conditions, the dominance of DMN is observed. During and after acute stress induction, a decrease in DMN FC and an increase in SN FC were reported (van Marle et al., 2010; Vaisvaser et al., 2013; Quaedflieg et al., 2015; van Oort et al., 2017). The activation of the SN has also been associated with spontaneous fluctuations in pupil dilatation and vigilance levels in the resting state (Schneider et al., 2016).

The changes in the association of HRV with the core brain networks in pre-and post-stress resting-state (Chand et al., 2020) conditions point towards a link to the organism’s vigilance state (Chand et al., 2021). Since the brain receives internal and external sensory inputs, processes them in the frame of previous information, and moderates the actions to maintain the organism in a state suitable for survival and continuous shifts between various functional states. In addition, due to slow fluctuations in cognitive, emotional, and autonomic functions, the flexibility of coupling between the different components of the psychophysiological system may serve the adaptive behavior (Young et al., 2017). Interestingly, the temporal association of the HRV has also been reported to covary with temporal changes in the functional connectivity related to vigilance (Chang et al., 2013, Schumann et al., 2021). The results of this thesis suggest that HRV is likely linked to an adaptive process in which a dynamic adaptation of the brain networks could adjust the psychological and physiological states by regulating vigilance state and reorienting attention (Herrman, Chang, et al., 2013, Young et al., 2017).

The dynamic nature of stress response

The first study showed that subjects not only had a higher mean vigilance level but also maintained a hyper-stable high vigilance state suggesting a dynamic nature of the stress response (Chand et al., 2021). These observations are corroborated by previous investigations showing the dynamic nature of stress responses (Hermans et al., 2014; Sousa, 2016). Each stress response system has specific spatiotemporal

profiles during and after the stress exposure. These unique spatiotemporal patterns enable an efficient response. For example, rapid actions of NE lead to an increase in alertness to provoke immediate changes in behavior. Delayed reactions to stress through corticosteroids enable a sustained, adaptive stress response. Since being aware of surrounding conditions during stressful or threatening situations is essential, subjects should maintain a high vigilance level under stress (Oken et al., 2006). In addition, vigilance levels also show unique temporal characteristics, which can help characterize some neuropsychiatric disorders (Olbrich et al., 2011, 2012). For example, patients with mania and attention-deficit/hyperactivity disorder exhibit an unstable vigilance regulation and show a rapid drop into lower vigilance stages during resting conditions, contrary to MDD, who exhibit a hyper stable vigilance pattern (Hegerl & Hensch, 2014). A healthy individual demonstrates adaptive vigilance regulation by showing the patterns of a progressive decline in vigilance levels during resting conditions.

Dynamic analysis pipelines also enable the examination of the temporal association patterns between two functions by providing additional time points other than a single summary value for the entire scan. The second study examined the minute-by-minute association between HRV and FC between canonical brain networks and showed that acute stress exposure alters the associated pattern between HRV and activity in brain networks (Chand et al., 2020). Therefore, implementing a multimodal approach provides the benefit of measuring stress interactions between stress systems rather than focusing on a single system. Due to different temporal response trajectories and unique structural properties of these systems, the response of one stress system may not coincide with the others. For example, many studies reported incongruent responses between cortisol levels, subjective ratings, and sympathetic activity indexes during stress (Campbell & Ehlert, 2012). According to the ‘stress coherence/compensation model’, the interaction among the stress response systems might represent a coherence between them that shows linear relationships and compensates for one another to achieve an optimized response (Andrews et al., 2013). A hypo/hyperactivation of one system might affect the activations of the other and shift various psychological states (Andrews et al., 2013). Earlier studies suggest that different stress response systems have their temporal trajectory dynamic, and

multimodal analysis of stress responses can maximize resolvable information that can be lost if the signal is analyzed time invariantly or separately (Lurie et al., 2020; Preti et al., 2017). Indeed, our findings in the second study showed that stress-induced changes in static FC between core brain networks are accompanied by changes in the minute-by-minute associations between the HRV and dFC between brain networks. Our findings support the conceptual premise that the brain and the heart function in a closely coordinated manner to maintain the homeostatic state of the organism in a constantly changing environment.

4. Conclusion

The study shows the promise of a multimodal approach to examining the relationship between different response systems. The acute psychological stress affects the brain dynamics by delaying the expected decline in vigilance levels and altering the temporal association pattern between HRV and FC between core brain networks even after the stressor has passed. This set forth that EEG-derived vigilance metrics can index stress responses and temporal dynamics of vigilance regulation. Secondly, the alteration in the temporal association pattern between HRV and FC between core brain networks might also serve as an index for the optimal response to stress. The proposed dynamic analysis methods offer great potential for a comprehensive understanding of the temporal properties of the stress response as well as among large-scale functional networks and heart rate variability, which will assist the diagnosis and therapeutics of individuals in need.

5. Future Outlook

In future studies, current findings can be complemented by using larger samples that represent a broader population with regard to including participants from both genders and who show a larger variation in susceptibility to stress.

The presented research focused on the carry-over effects of stress. The stress response can also be divided into various phases, including the anticipation period, during the actual stress and recovery phase (Kühnel et al., 2021). Each phase of the

stress response has different effects on the risk of stress-related disorders. The presented research focused on the carry-over effects of stress. Although studying post-stress effects in humans may contribute to understanding psychological trauma etiology in its early phases (van Marle et al., 2010b), the investigation of the interaction between central and peripheral stress responses during the stress tasks would complement the current findings.

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List of Publications

Chand, Tara, Sarah Alizadeh, Hamidreza Jamalabadi, Luisa Herrmann, Marina Krylova, Galina Surova, Johan van der Meer, Gerd Wagner, Veronika Engert, and Martin Walter. "EEG revealed improved vigilance regulation after stress exposure under Nx4–A randomized, placebo-controlled, double-blind, cross-over trial." IBRO neuroscience reports 11 (2021): 175-182.

Chand, Tara, Meng Li, Hamidreza Jamalabadi, Gerd Wagner, Anton Lord, Sarah Alizadeh, Lena V. Danyeli, Luisa Herrmann, Martin Walter, and Zumrut D. Sen. "Heart rate variability as an index of differential brain dynamics at rest and after acute stress induction." Frontiers in neuroscience 14 (2020): 645.

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Delineation of contribution to collective work

Chand, Tara, Sarah Alizadeh, Hamidreza Jamalabadi, Luisa Herrmann, Marina Krylova, Galina Surova, Johan van der Meer, Gerd Wagner, Veronika Engert, and Martin Walter. "EEG revealed improved vigilance regulation after stress exposure under Nx4–A randomized, placebo-controlled, double-blind, cross-over trial." IBRO neuroscience reports 11 (2021): 175-182.

M.W conceived the experiments. J.V.M. conducted the experiments. S.A., H.J., and M.K. preprocessed resting-state EEG data. G.S., and M.K. implemented VIGALL algorithm on EEG data. T.C. , S.A., H.J., and M.K. analyzed the results. T.C., S.A., L.H., and H.J wrote the manuscript and all authors reviewed interim drafts and final version of the manuscript

Chand, Tara, Meng Li, Hamidreza Jamalabadi, Gerd Wagner, Anton Lord, Sarah Alizadeh, Lena V. Danyeli, Luisa Herrmann, Martin Walter, and Zumrut D. Sen. "Heart rate variability as an index of differential brain dynamics at rest and after acute stress induction." Frontiers in neuroscience 14 (2020): 645

TC, ZDS, SA, LH, HJ, and MW were involved in the development of the study. TC, ML, GW, AL, LD, MW, and ZDS were involved in the analysis and interpretation of data. All authors gave approval of the final version and agreed to be accountable for all aspects of the work

Acknowledgements

I am wholeheartedly thankful to my supervisor, Prof. Dr. med. Martin Walter. His scientific mentorship, constant support, optimism, and outstanding training have helped me to advance my scientific career. I am wholeheartedly thankful to Prof. Dr. Jennifer Svaldi for her critical feedback on my research work which enabled me to advance my scientific aptitude.

I would further like to extend my sincere thanks to my colleagues, especially Dr. Duygu Zümurt Sen and Dr. Meng Li, for their excellent and timely guidance on various experiments and social aspects of life. I would also like to thank you for taking out their valuable time to help and carry out numerous analyses.

I am wholeheartedly thankful to my officemates, Nooshin Javaharipour, Melanni Nani Zapeda, Nikolai Blekic, for providing a great office environment and always being ready for scientific and non-scientific discussion. I extend my special thanks to the members of the group specially.

I am thankful to Dr Neeraj Upadhyay, Dr. Vinod Kumar, and Dr. Parveen Kumar for proofreading my thesis. I am also thankful to Lena Daniely for translating the summary of the thesis into German.

I express my gratitude to all my friends, in and out of Tübingen, for making my time in Germany enjoyable and memorable.

Finally, my deepest gratitude to my parents, my partner Neha Laduna, my daughter Navya Shree, and other members of the family for their love, help, and support. I am thankful for their sacrifices to promote my education.

IBRO Neuroscience Reports 11 (2021) 175–182

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

Research Paper

EEG revealed improved vigilance regulation after stress exposure under Nx4 – A randomized, placebo-controlled, double-blind, cross-over trial

Tara Chand [a,c,1], Sarah Alizadeh [a,c,1], Hamidreza Jamalabadi [c,f], Luisa Herrmann [a,c], Marina Krylova [a,c], Galina Surova [d], Johan van der Meer [b,g], Gerd Wagner [a], Veronika Engert [e], Martin Walter [a,c,*]

[a] Department of Psychiatry and Psychotherapy, Jena University Hospital, Jena 07743, Germany
[b] QIMR Berghofer Medical Research Institute, Brisbane 4006, Australia
[c] Department of Psychiatry and Psychotherapy, University of Tübingen, Tübingen 72076, Germany
[d] Department of Psychiatry and Psychotherapy, Leipzig University Medical Center, Leipzig 04103, Germany
[e] Institute of Psychosocial Medicine, Psychotherapy and Psychooncology, Jena University Hospital, Jena 07443, Germany
[f] Department of Psychiatry and Psychotherapy, Philipps-Universität Marburg, Marburg, Germany
[g] Amsterdam UMC, Department of Radiology and Nuclear Medicine, 1105AZ, Amsterdam, The Netherlands

ARTICLE INFO

Keywords:
Psychosocial stress
Vigilance
Resting-state
EEG
Neurexan

ABSTRACT

Objectives: Vigilance is characterized by alertness and sustained attention. The hyper-vigilance states are indicators of stress experience in the resting brain. Neurexan (Nx4) has been shown to modulate the neuroendocrine stress response. Here, we hypothesized that the intake of Nx4 would alter brain vigilance states at rest.
Method: In this post-hoc analysis of the NEURIM study, EEG recordings of three, 12 min resting-state conditions in 39 healthy male volunteers were examined in a randomized, placebo-controlled, double-blind, cross-over clinical trial. EEG was recorded at three resting-state sessions: at baseline (RS0), after single-dose treatment with Nx4 or placebo (RS1), and subsequently after a psychosocial stress task (RS2). During each resting-state session, each 2-s segment of the consecutive EEG epochs was classified into one of seven different brain states along a wake-sleep continuum using the VIGALL 2.1 algorithm.
Results: In the post-stress resting-state, subjects exhibited a hyper-stable vigilance regulation characterized by an increase in the mean vigilance level and by more rigidity in the higher vigilance states for a longer period of time. Importantly, Nx4-treated participants exhibited significantly lower mean vigilance level compared to placebo-treated ones. Also, Nx4- compared to placebo-treated participants spent comparably less time in higher vigilance states and more time in lower vigilance states in the post-stress resting-state.
Conclusion: Study participants showed a significantly lower mean vigilance level in the post-stress resting-state condition and tended to stay longer in lower vigilance states after treatment with Nx4. These findings support the known stress attenuation effect of Nx4.

1. Introduction

Neurexan (Nx4) is a medicinal over-the-counter product in Germany which contains Avena sativa, Coffea arabica, Passiflora incarnata and Zincum isovalerianicum. It is approved by the German Federal Institute for Drugs and Medical Devices (German: Bundesinstitut für Arzneimittel und Medizinprodukte [BfArM]) for the treatment of sleep disorders and restlessness. Its effects have been demonstrated in several human (Doering et al., 2016; Herrmann et al., 2020; Hübner et al., 2009; Waldschütz and Klein, 2008) and rodent studies (Dimpfel, et al., 2012). Thus, the intake of Nx4 for 28 days was shown to improve sleep quality by increasing sleep duration and reducing sleep latency as reported in patients' sleep diaries over 14 days, and at the end of the study (Waldschütz and Klein, 2008). Taking Nx4 for two weeks led to a reduction in nervousness and restlessness in patients visiting German general practitioners (Hübner et al., 2009). A single dose of Nx4 (6 tablets over 2.5 h)

* Correspondence to: Department of Psychiatry and Psychotherapy, Jena University Hospital, Philosophenweg 3, Jena 07743, Germany.
E-mail address: Martin.Walter@med.uni-jena.de (M. Walter).
[1] Equal contribution.

https://doi.org/10.1016/j.ibneur.2021.09.002
Received 19 March 2021; Received in revised form 13 August 2021; Accepted 18 September 2021
Available online 25 September 2021

reduced the acute neuroendocrine stress response measured in terms of stress induced changes in salivary cortisol and plasma adrenaline levels in healthy volunteers (Doering, Wegner et al. 2016). Studies of brain electrophysiology measured via electroencephalography (EEG) suggest a calming effect of Nx4. In rats, the drug increased the low-frequency delta and theta brainwaves (Dimpfel, et al., 2012). In humans, elevated frontotemporal beta2 power, a surrogate parameter of stress-induced anxiety, was dampened by the drug (Dimpfel, 2019). Further investigations are necessary to unravel the mode of action of Nx4 and better understand its beneficial effect on the acute stress response in humans.

Several behavioral states such as mood, stress, arousal, and vigilance, are determined by internal and external (environmental) stimuli such as motivationally salient or threatening stimuli, which are processed in the body by the autonomic nervous system and result in the release of specific hormones into the bloodstream. The immediate stress response in the body is characterized by the activation of both the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of catecholamines (adrenaline and noradrenaline) and corticosteroids. In the brain, particularly the noradrenaline producing locus coerueus (LC) and amygdala are playing a central role in the processing of salient information and mediating the effects of stress, e.g. due to threatening stimuli by triggering ANS response to stressors. The noradrenaline released by LC neurons subsequently modulates the stress or arousal response in frontal brain regions and modulates the activity of amygdala, inducing a rapid increase of attention and/or perception to/of a stimulus and changing the cortical arousal and vigilance level (Sara and Bouret, 2012, van Marle, et al., 2010; Veer, et al., 2011). In sum, the LC plays a central role in the induction and regulation of cortical arousal (Berridge, 2008 for comprehensive review) and induces a higher vigilance state under stress, a state referred to as tonic alertness by Oken et al. (2006). This higher vigilance state leads to a state of elevated sustained attention and alertness, and drives the stress response in associated brain regions including a faster processing of visual stimuli.

EEG is a reliable tool to investigate changes in vigilance states of the brain and can be used as a marker of cortical arousal. Brain vigilance states were shown to be altered by affective stimuli (Borchardt, et al., 2018), by stress and in the context of stress-related psychopathology (Klumpp and Amir, 2009; Silvers, et al., 2017). For example, a significantly higher and prolonged vigilance level, and rigid, hyper-stable regulation of vigilance during rest were found in patients with obsessive-compulsive disorder (OCD) compared to healthy controls (Olbrich, et al. 2013). Investigating the effect of three attachment narratives on listeners' cortical arousal, Borchardt et al. (2018) found that the high vigilance state caused by listening to any of the narratives decreased faster after an insecure-dismissing narrative than after insecure-preoccupied and secure narratives. Henckens et al. (2015) showed that neural vigilance processing varied depending on basal cortisol levels and stress-induced cortisol release following acute stress exposure. In detail, the authors demonstrated that acute stress affected the vigilance neurocircuitry, and that individual differences in neural stress responses were associated with differences in basal and stress-induced cortisol levels.

Staying aware of ones environment during stress exposure can be necessary for survival. Therefore, stress experience is characterized by a hyper-vigilance state, involving increased cortical arousal, tonic alertness, maintaining attention towards the external environment and fast responsiveness. A state of rest is contrarily characterized by a reduced vigilance state and cortical arousal (Olbrich, et al., 2013).

In the current study, vigilance states were estimated with an EEG-based algorithm (Vigilance Algorithm Leipzig, VIGALL 2.1) (Hegerl, et al., 2012; 2017) which uses the cortical current density activity to classify different states of vigilance from high wakefulness to drowsiness until sleep onset during resting-state. We hypothesized that the participant group receiving Nx4 would show a lower resting-state vigilance following stress exposure than the placebo control group. To test this hypothesis, we investigated how the stress-induced vigilance regulation changed in resting-state EEGs before and after the intake of either Nx4 or placebo.

2. Methods

The current study is a post-hoc analysis from the NEURIM trial registered at ClinicalTrials.gov under the number NCT02602275 (date of registration: 28/10/2015), and approved by the ethics committee of the University of Magdeburg as well as the Competent Authority (Federal Institute for Drugs and Medical Devices). It complies with the ethical principles of the 1996 Declaration of Helsinki (Somerset West, Republic of South Africa), principles of the Good Clinical Practice (GCP) provided in the International Conference on Harmonisation (ICH) Harmonized Tripartite Guidelines for GCP 1996, and all applicable national laws and regulations. Prior to study participation, written informed consent was given by each participant after receiving detailed information on the study protocol from the principal investigator. The study took place at the Medical Faculty of the Otto-von-Guericke University of Magdeburg, Germany, from August 18th, 2015 to December 3rd, 2015. EEG and fMRI acquisitions were conducted at the university's local imaging center. Participants who fulfilled the inclusion criteria were recruited from July to November 2015. All screenings and measurements were collected between August and December 2015.

2.1. Trial design

The NEURIM trial was conducted as a randomized, placebo-controlled, double-blind, two-period cross-over trial with an explorative design and confirmatory principles. Each participant attended three visits (Fig. 1A): On Day 0, medical and psychological symptoms and compatibility for MRI were screened. Psychometric testing of personality traits, life experiences and sensitivities were completed between 3 and 7 days prior to Day 1. Day 1 comprised the randomized distribution of participants to a respective treatment group, fMRI and EEG data acquisition. Considering the washout period, a cross-over EEG-fMRI session (Day 2) took place after 7–35 days from Day 1. Apart from the type of Investigational Medicinal Product (IMP), i.e., active ingredient versus placebo, study procedures were identical on both days (Day 1 and Day 2). Participants who received verum on Day 1 were given placebo on Day 2 and vice versa. Additional information of active ingredients in verum may be seen in Supplementary Table S1.

All resting state EEG measurements were acquired simultaneous with fMRI in two sessions (before and after the intake of IMP). All subjects were instructed to keep their eyes closed and do not engage in any specific tasks while trying not to fall asleep. A 12-minute baseline resting-state (RS0) was acquired before the intake of the IMP (Fig. 1B). Each participant got a single dose (three tablets) of either Nx4 or placebo 40–60 min before the second scan. During the interval between the first and the second resting-state scan (RS1 and RS2 respectively) EEG recordings were acquired while the participant performed two computerized tests, the Attention Modulation by Salience Task (AMST) (Dinica et al., 2016) and an auditory oddball task (Segalowitz and Barnes, 1993). The second scanning session comprised three tasks: 1) the Hariri task (Hariri et al., 2002), 2) the Expectancy task (Teckentrup et al., 2019), and 3) the ScanSTRESS task (a shortened version of the ScanSTRESS by Streit et al. 2014), as well as pre- (RS1) and post-task (RS2) eyes-closed resting-state scans of 12 min each. Psychometric tests measuring anxiety and mood were completed before and after the first and second scans. All fMRI measurements were realized with simultaneous EEG data acquisition.

To induce psychosocial stress, a shortened version of the ScanSTRESS task (Streit, Haddad et al. 2014), which is an adapted version of the Montreal Imaging Stress Task (Dedovic et al., 2005), was used. This paradigm consists of two runs of mental rotation and arithmetic

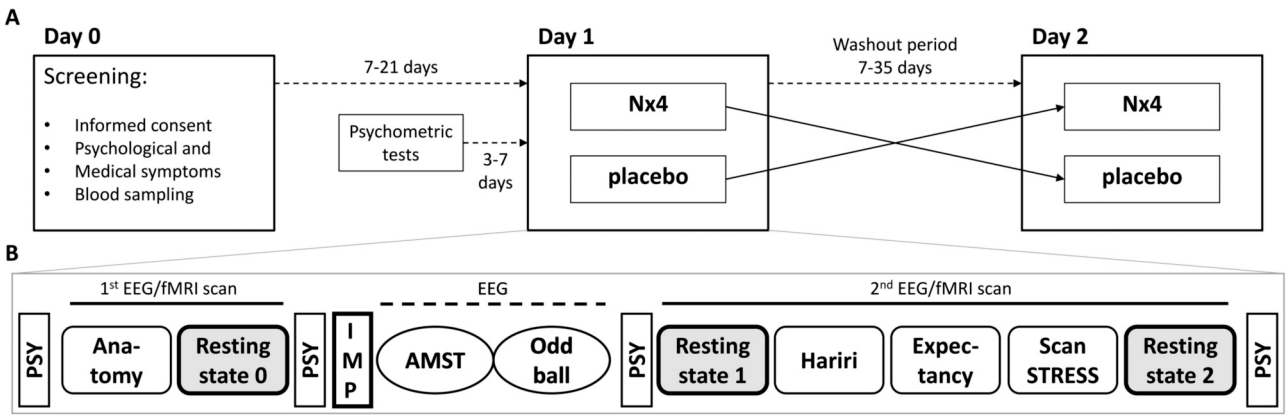

Fig. 1. Design of the randomized, placebo-controlled, double-blind, two-period cross-over trial. A) Overall study design. On Day 0, participants gave informed consent, and psychological and medical symptoms were screened. IMP administration and EEG/fMRI measurements took place on each of both cross-over periods (Day 1 and Day 2). A minimum of 7 and a maximum of 21 days elapsed between Day 0 and Day 1. During this time and 3–7 days prior to Day 1, participants completed a battery of psychometric tests. The washout period between Day 1 and Day 2 amounted to 7–35 days. B) Study design of the cross-over EEG/fMRI sessions (as described in Herrmann et al., 2020). EEG/fMRI acquisition began with an anatomical scan followed by a baseline resting-state measurement. After intake of the IMP, the two EEG paradigms (AMST and Oddball) were performed. The second EEG/fMRI scan was comprised of three tasks, including stress induction via the ScanSTRESS paradigm, and two resting-state measurements before and after the tasks, respectively. Psychometric tests assessing anxiety and mood were completed before and after the first and second scans. PSY = Psychometric Tests; fMRI = functional Magnetic Resonance Imaging; IMP = Investigational Medicinal Product; EEG = Electroencephalography; AMST = Attention Modulation by Salience Task.

calculation tasks, both of which include stress and control blocks. In the stress blocks, participants receive negative social evaluative feedback and perform difficult excercises under time pressure. Excercises in the control blocks are provided without negative feedback and are overall less demeaning than those of the stress condition.

2.2. Participants and inclusion criteria

Forty healthy male volunteers, between the ages of 31 and 59 (mean: 43.74 ± 9.81 years) were recruited. Volunteers were only included if they were physically healthy based on laboratory tests and a physical examination, MRI compatible, nonsmoking, and fluent in German. Subjects were screened for ongoing psychological diseases or episodes using the Structured Clinical Interview for DSM-IV (Wittchen et al., 1997). Chronic stress levels were determined using the screening version of the Trier Inventory for Chronic Stress (TICS; Schulz and Schlotz (1999)), and the Perceived Stress Scale (PSS; S. Cohen, Kamarck, and Mermelstein (1983)). Participants scoring within the range of mild to moderate stress (TICS Score ≥9 and ≤36, PSS > 9) were included. All others were excluded to ensure that participants were in principle

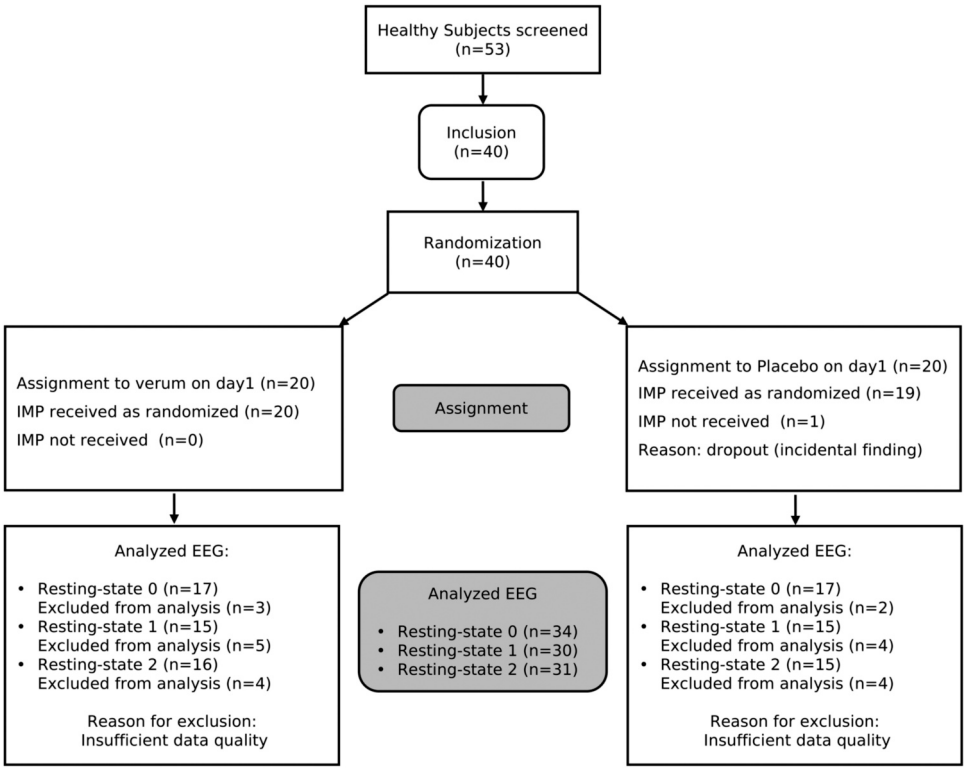

Fig. 2. CONSORT flow diagram showing number of participants through each stage of the randomized cross-over trial.

susceptible to stress, and to avoid a ceiling effect of stress activity in cases of current severe or chronic stress experience. All subjects gave written informed consent before participation. Because during baseline MR measurements, one subject was excluded due to an incidental finding, 39 subjects were included in this study. Out of those 39 participants, only subjects with good-quality EEG data after preprocessing from both experimental days were included in the statistical analysis of each resting-state, resulting in 34, 30 and 31 subjects in baseline (RS0), pre-stress (RS1) and post-stress (RS2) resting-states, respectively.The flow of participants through each stage of the trial according to the CONSORT diagram is depicted in Fig. 2.

2.3. Blinding and randomization

Investigators and participants were blinded during the study period. Because verum and placebo contained lactose monohydrate and magnesium stearate, they were identical in taste, size, color, and labeling. As mentioned in the details of unblinding safety management, an investigator was given authority to break the treatment code in case of emergency only. There was no emergency unblinding reported during the study. A statistician not involved in the study generated the randomization codes within IDV Gauting, providing biometric services and strategic consulting. The randomization codes and the whole procedure were filed at a secure place by IDV Gauting until the study database was closed. Following the randomization list, a non-involved statistician also provided pack lists with medication numbers to Heel GmbH for manufacturing of the drug kits. A random allocation of participants to Nx4 or the placebo group was performed in a 1:1 randomization. A corresponding random code list was prepared, using the randomly permuted block scheme with a fixed block size. Participants were enrolled based on the randomization number recorded on the study medication. The next participant eligible for randomization was allocated to the lowest available randomization number at the site. A validated program RANCODE (Ver. 3.6) was used to prepare the sealed random code list and the sets of sealed envelopes in a standard working environment at idv Data Analysis and Study Planning, Krailling, Germany.

2.4. EEG data acquisition

In resting-state scans, EEG and fMRI data were acquired simultaneously with a Brain Products Easy Cap with 64-channels. All the subjects were instructed to keep their eyes closed and do not engage in any specific tasks while trying not to fall asleep. FCz and CPz electrodes were placed as reference and ground electrodes, respectively. One channel was placed on the subject's back and was used for ECG detection. The data were recorded using BrainVision Recorder Professional V.1.20.0801 with a sampling rate of 5000 Hz. To increase the quality of the EEG recordings assessed in the simultaneous EEG-fMRI scans, the EEG cap was augmented with six carbon-wire loops (CWLs). Four CWLs were placed on the outer surface of the EEG cap at the left and right frontal and the left and right posterior locations. Two CWLs were attached to the cables connecting the EEG cap to the EEG amplifier (BrainAmpMR Plus). For further details on CWLs, see van der Meer et al. (2016).

2.5. EEG data preprocessing

To clean the resting-state EEG data from MRI gradient artifacts, we employed a state-of-the-art motion informed template subtraction method realized by the Bergen EEG-fMRI toolbox (Moosmann et al., 2009). For averaging and to obtain an MRI template waveform, 25 MRI artifacts in a sliding window were used (Allen et al., 2000). We used the realignment parameters from the fMRI analysis as interruption points, i.e. whenever the displacement vector reached a threshold value of 0.5 mm the window buffer was reset. After bandpass filtering of the artifact-corrected EEG data from 0.3 Hz to 200 Hz and downsampling to 1000 Hz, the helium pump and ballistocardiographic (BCG) artifacts were removed using the novel carbon-wire loop-based artifact correction technique (van der Meer, et al., 2016). This toolbox regresses out any EEG artifact which is correlated with the movements recorded by each of the six CWLs. Here we used Hann tapers with overlapping windows of 6 s length and a delay embedding of 21 ms as recommended by van der Meer, Pampel et al. (2016). Next, the data were segmented into 2 s epochs (i.e. equivalent to the repeation time (TR) of the BOLD resting-state scans) and the epochs containing muscle and head movements artifacts were removed from the data set in a semiautomatic process using custom MATLAB scripts. Channels that contained too many epochs with artifacts were also removed and interpolated using routines provided by EEGLAB (Delorme and Makeig, 2004). The final step of artifact rejection included the independent component analysis (ICA) decomposition of the EEG data and removing the components that reflected eye movements, heartbeat, continuous muscle activity and residuals of fMRI-artifacts.

2.6. Time course of vigilance regulation

To extract the time course of vigilance fluctuations and verify how the regulation of brain arousal changed after treatment with Nx4 compared to placebo, we performed an EEG-based vigilance classification algorithm on resting-state EEG data using the Vigilance Algorithm Leipzig (VIGALL 2.1; Hegerl, et al., 2017). The VIGALL 2.1 is a freely available Add-In for BrainVision Analyser 2.1 (Brain Products GmbH, Gilching, Germany). The algorithm requires several standardized pre-processing steps including filtering (0.5–70 Hz, 50 Hz Notch, 48 dB Slope), downsampling (100 Hz) and manual marking the indicators of sleep onset (sleep spindles and K-complexes), as these markers are used by VIGALL to classify C-stages (for detailed description please see the manual: Hegerl et al., 2017). The VIGALL classification is based on the distribution of cortical current density activity over four distinct regions of interest (occipital, parietal, temporal, frontal). An estimate of the cortical current density is separately computed for the delta/theta and the alpha frequency range using the LORETA method (Pascual-Marqui, et al., 1994). The delta/theta band was set between 3 and 7 Hz. Each subject's individual alpha center frequency was automatically detected, and the alpha band was defined as ± 2 Hz around the individual alpha center frequency. Vigilance states in 355 consecutive EEG epochs (each had a duration of 2 s) were estimated in each resting-state (RS0/RS1/RS2) and condition (verum/placebo). This was the maximum length of recording available for each subject. Each segment was automatically classified into one of seven different brain states along a wake-sleep continuum (alertness: 0, A1, A2, A3; drowsiness: B1, B2/3; and sleep onset: C). Stage 0 is characterized by low-amplitude non-alpha EEG. In stages A1-A3 dominant alpha activity is prevalent in occipital (A1), central-frontal (A2) and frontal (A3) areas. Stage B1 reflects low-amplitude non-alpha EEG with slow eye movements. In stage B2/3 dominant delta and theta powers are observed. Sleep onset stage C is characterized by the appearance of sleep spindles and/or K-complexes. Because electrooculography was not available, differences of the signals between two frontal electrodes F7 and F8 were used to estimate horizontal eye movements. To compare the time course of vigilance fluctuations, we assigned a vigilance score ranging from 1 to 7 to each EEG-vigilance state (1 represents the lowest (stage C) and 7 the highest (stage 0) vigilance state), and averaged the vigilance scores within non-overlapping 2-min intervals, resulting in 6 time points (equivalent to 12 min) for each vigilance curve.

2.7. Statistical analysis

To examine the drug effect on the time course of vigilance fluctuations, a repeated-measures ANOVA with two main factors of treatment (verum vs. placebo) and time (6 different time points in the vigilance

curve) as within-subject factors and treatment-sequence (placebo-verum or verum-placebo) as a between-subject factor was performed in each resting-state. The Greenhouse-Geisser correction was applied if the sphericity assumption was violated. To examine the drug effect on the slope and offset of the vigilance curve, a line was fitted to the vigilance curve of each subject using linear regression analysis. The resulting slopes/offsets were compared across subjects using paired T-tests. Because the data was not normally distributed, percentages of vigilance states under Nx4 and placebo were compared using the two-sided Wilcoxon signed-ranked test. Only those subjects that had good-quality EEG data after preprocessing from both experimental days were included in the statistical analysis of each resting-state, resulting in 34, 30 and 31 subjects in RS0, RS1 and RS2, respectively.

3. Results

3.1. Temporal dynamics of vigilance levels before and after stress exposure

The time course of EEG vigilance fluctuations averaged over subjects in the resting-state recordings during baseline (RS0), before, and after exposure to stress (RS1 and RS2 respectively) is shown for verum and placebo conditions separately in Fig. 3. During RS0 and RS1, subjects exhibit progressive decline to lower vigilance levels over the time of scanning (Fig. 3C and D). In contrast, subjects continuously remain in high arousal states (hyper-stable arousal regulation) during RS2 after the stress task (Fig. 3E).

To quantify the general effect of stress on the slope and offset of the vigilance curve, we performed paired *t*-tests to compare data before and after stress from the placebo session only (RS2 vs. RS1). We found that acute stress induction significantly decreased the steepness [$t(29) = 3.82$, $p = 0.0005$] and increased the offset [$t(29) = 2.23$, $p = 0.032$] of the vigilance curve.

3.2. Changes in slope and offset of vigilance curves after the treatment with Nx4

Repeated-measures ANOVA with within-subject main factors of treatment (verum vs. placebo) and time (6 different time points of the vigilance curve) in each resting-state showed significant main effects of treatment ($F(1,29) = 6.53$, $p = 0.016$) and time ($F(2.52,29) = 3.26$, $p = 0.033$, after Greenhouse-Geisser correction) on the vigilance score only in post-stress resting-state (RS2). No significant carry-over effect (treatment × treatment-sequence interaction: $F(1) = 0.318$, $p = 0.58$) or treatment × time interaction ($F(1,3.43) = 0.352$, $p = 0.814$) was found. Wilcoxon test for paired samples confirmed that Nx4 significantly reduced the mean/overall vigilance level of subjects only in RS2 ($Z = -2.645$, $p = 0.008$). No significant result was found in RS1 or RS0. Repeated-measures ANOVA with main factors of treatment (verum vs. placebo) and resting-state (RS1 vs. RS2) showed no significant treatment or treatment x resting-state interaction.

Testing for the effect of Nx4 on the slope and offset of the vigilance curves using paired *t*-tests showed that Nx4 reduced the offset of the vigilance curve after stress (trend level: $t(30) = -1.89$, $p = 0.068$), but had no significant effect on the slope of the curve ($t(30) = 0.315$, $p = 0.755$). No significant drug effect was found on the slope or offset of the vigilance curve in RS1 or RS0.

3.3. Prevalence of different vigilance stages

Comparing the percentage of EEG-vigilance stages 0, A1, A2, A3, B1 and B2/3 in the verum vs. placebo conditions using Wilcoxon signed-rank test revealed significant differences between Nx4 and placebo only for B2/3 in RS2. The percentage of EEG segments with the low vigilance stage B2/3 was significantly higher under Nx4 compared to placebo (mean in %±SD: Nx4 = 11.02 ± 21.17, placebo = 3.76 ± 8.70; $p = 0.006$). No significant difference was found in RS1 or RS0.

3.4. Behavioral correlations with stress-induced changes in mean vigilance

We further analyzed the associations of the stress-induced changes in mean vigilance with perceived stress scale measures like TICS and PSS-10. To do this, we compared the stress-induced changes in vigilance (RS2-RS1) with the PSS-10 and TICS in the placebo and verum groups separately. No significant correlation between TICS and the delta mean vigilance (RS2-RS1) in the verum or placebo groups was found. However, we found that the stress-induced changes in mean vigilance is positively correlated with PSS-10 scores in the placebo group ($R = 0.46$, $p = 0.02$, see Fig. 4). This means that subjects with higher perceived stress scale based on PSS-10 have more increase of the mean vigilance after stress induction if they are treated with placebo. There is no correlation when subjects are treated with Nx4 (See below Fig. 4).

4. Discussion

EEG signals act as a marker of arousal regulation in the brain. In the current study, we showed that placebo-treated control subjects

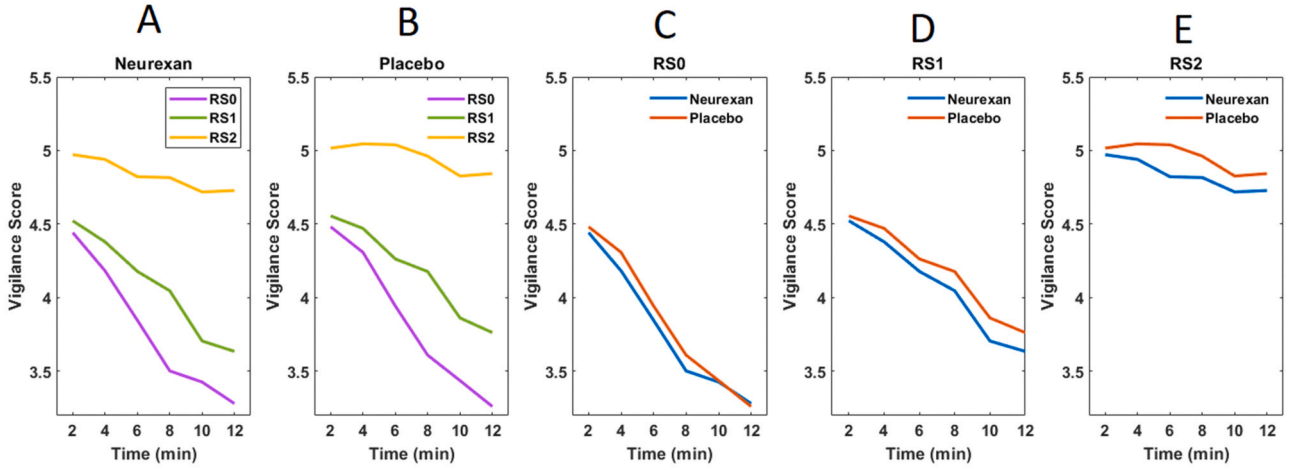

Fig. 3. Time course of EEG-vigilance fluctuations under Neurexan (A) and placebo (B) in three different resting-state recordings. For the sake of comparison, vigilance curves under Neurexan and placebo are shown in each resting-state recording separately (C-E). Each of the six time points of the curve corresponds to the average vigilance score in a 2-min window.

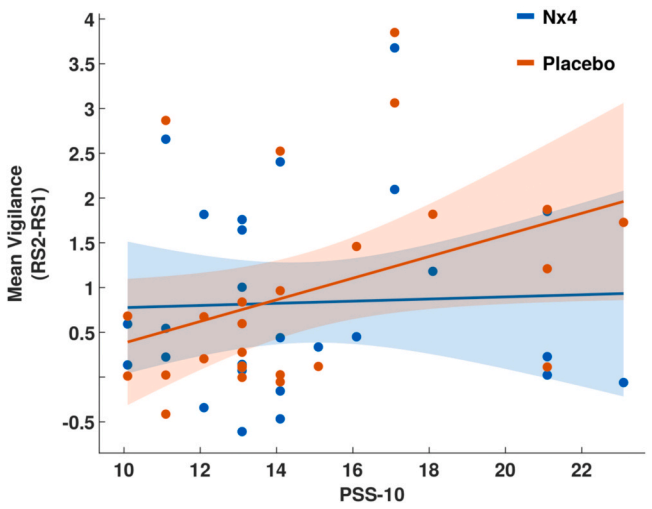

Fig. 4. The relationship between the stress induced changes in mean vigilance with the perceived stress scale measured by PSS-10 in placebo group (red) and Nx4 group (blue). The correlation is significant only in placebo condition ($R = 0.46$, $p = 0.02$).

exhibited a hyper-vigilance state characterized by an increase in the mean vigilance level and by more rigidity in the higher vigilance states for a longer period of time after acute psychosocial stress experience. In other words, during the resting-state following acute stress exposure (RS2), participants were more alert and tense, and showed a hyper-stable vigilance regulation in the placebo condition. This state was quantified in terms of an increase in the offset and a decrease in the steepness of the vigilance curve in post– compared to pre-stress conditions. Subjects treated with the over-the-counter product Neurexan (Nx4) as compared to placebo had significantly lower mean vigilance levels, spent significantly more time in the lower vigilance state B2/3 and exhibited a decrease in the offset of their vigilance regulation after stress. These findings show that Nx4 reduces the effects of stress on vigilance regulation by decreasing the offset (but not the slope) of the vigilance curve, thus indicating a stress attenuating effect of Nx4.

In the pre-stress resting-state measurements (RS0 and RS1), subjects showed a steeper decline in the vigilance state as compared to after the acute stress task (RS2). Further, we found a significant decrease in the steepness and increase in the offset after the stress task (RS2). This shows a hyper-stable arousal regulation after the stress task and represents a significant stress effect on arousal regulation.

Considering the treatment effect, a significant decrease in the offset but not the steepness of the vigilance curve in post-stress resting-state (RS2) under Nx4 as compared to placebo suggests that Nx4 does not change the dynamics of the vigilance regulation over time (slope), but rather decreases the mean vigilance level by attenuating the stress response and calming the subject. Interestingly, no significant drug effect was observed in RS1. In the pre-stress condition, both placebo and drug group showed a typical vigilance decline in the scanner (Tagliazucchi & Laufs, 2014). Absence of a significant difference between Nx4 and placebo in RS1 indicates that reduction in the mean vigilance level after stress under Nx4 was not due to a sleep-inducing effect but rather a stress-attenuating effect of Nx4.

The change in stress response under Nx4 may relate to its effect on amygdala activity. Activation of the amygdala and an increase in the vigilance level help to quickly react to threatening situations (Klumpp and Amir, 2009). In a similar line of argument, previous studies showed increased amygdala activity in stress-related disorders (Drevets, 2003; Etkin and Wager, 2007). Interestingly, recent investigations in the current participant sample (Herrmann et al., 2020) showed lower activity in the centromedial amygdala (CeMA) response to negative stimuli (emotional face matching, Hariri task) in the Nx4 compared to the placebo group. The CeMA has efferent projections to cholinergic neurons (Davis and Whalen, 2001; Fadok et al., 2018). Via these projections, amygdala activation indirectly affects the whole cortex thus playing an important role in modulating general arousal (Davis and Whalen, 2001). Furthermore, the CeMA-thalamus coupling helps to maintain sustained attention on potential threats and modulates the vigilance level (Torrisi et al., 2018, Cain et al. 2002, Fadok et al., 2018). Taken together, our results suggest that the Nx4-induced alteration of the vigilance level might be mediated by the amygdala which is critically involved in the stress response by altering the vigilance level.

Schulz et al. (1998) previously tested Valeriana officinalis and Passiflora incarnata for their sleep-inducing effects and the capacity to compensate the stimulating effect of caffeine using self-report ratings and quantitative EEG (qEEG) in a placebo-controlled study with female subjects. They found Valeriana officinalis to show a significant effect on the subjective ratings of sedative effects. However, qEEG could not reliably predict sedation. Nevertheless, the drugs affected the qEEG signals. Valeriana officinalis increased delta, theta and slow alpha bands, whereas Passiflora incarnata only increased theta power. qEEG was also used by Schellenberg et al. (1993) who found that a phytopharmacological drug containing Radicis valerianae and Herbae passiflorae incarnatae increased theta- and alpha-power after two weeks of treatment in psychosomatic and affective patients. Similar to these findings, we found that the Nx4 group spent more time in the low vigilance state B2/3 in the post-stress resting-state, which represents dominant delta and theta power. Importantly, spending more time in lower vigilance states under Nx4 does not necessarily mean more drowsiness or loss of attention in the Nx4 group. In contrast, our results show no significant difference in arousal regulation (i.e., no change in the offset or slope of the vigilance curve) after Nx4 compared to placebo intake in the pre-stress resting-state condition (RS1). In fact, the modulating effect of Nx4 on vigilance regulation is evident only in the post-stress resting-state condition (RS2). Additionally, the stress-induced changes in vigilance show a positive correlation with PSS-10 only in the placebo group, suggesting that participants with higher perceived stress scale based on PSS-10 more increases in vigilance level after stress induction if they are treated with placebo. Interestingly, there is no correlation if participants are treated with Nx4 which could be due to stress-reducing effect of Nx4. Taken together, our results suggest that the stress-reducing effect of Nx4 may be due to its calming influence without deteriorating attention and concentration.

Several limitations of the present study should me mentioned. As no electrooculography was available during the recording, horizontal eye movements were estimated to consider this correction factor. Also, the EEG was acquired inside the MR scanner, which made it necessary to introduce additional corrections to the data. Lastly, since our sample is comprised only of healthy, middle-aged and mildly to moderately stressed male subjects, the generalizability of the current results to diseased patients should be considered with caution.

5. Conclusion

We found that in the post-stress resting-state, subjects exhibit a hyper-vigilance state (quantified as a rigid, hyper-stable vigilance curve), and their vigilance regulation does not show the decline typically observed during resting-state. However, in the resting-state following psychosocial stress, subjects under Nx4 exhibited significantly lower mean vigilance levels, spent significantly more time in lower vigilance states and exhibited a decrease in the offset of their vigilance regulation when compared to a matched placebo group. These findings indicate that Nx4 brings the post-stress vigilance regulation to a normal decline due to its stress-attenuating effects. However, the effect of Nx4 on chronically stressed individuals has yet to be investigated. Furthermore, in addition to the effect of Nx4, psychotherapy and behavioral modification might be very important to treat/prevent increased perceived psychosocial stress. However, this is out of the scope

of this study and may be investigated in future studies.

Declaration of competing interests

M.W. received institutional research support from Heel paid to his institution for this study, and from BrainWaveBank, H. Lundbeck A/S and LivaNova Belgium N.V., LivaNova PLC outside the submitted work. The University of Tübingen received institutional fees for advisory services by Prof. Walter from Heel GmbH, Servier Deutschland GmbH, Bayer AG and Janssen-Cilag GmbH. The University of Tübingen received financial support for conference attendance of T.C., S.A., L.H., M.K. and H.J. from Heel for presenting data of this study not reported in this article. L.H., J.V.M., T.C., S.A., H.J., and M.K. were part of M.W. team for this study, and declare no other conflict of interest outside the submitted work. All investigators followed the institutional guidelines for COI management in full compliance with the regulations of the Otto v. Guericke University, Magdeburg.

Acknowledgment

This study was funded by Heel GmbH (Heel), Baden-Baden, Germany, the manufacturer of Neurexan®. Heel had full administrative responsibility for conducting the study and collaborated with academic advisors in designing the study protocol, collecting and analyzing the data, and interpreting the results. During the study Heel has organized and chaired several expert review meetings. Centrial GmbH (Tübingen), a contract research organization, was employed by Heel to support study administration. Idv Datenanalyse & Versuchsplanung company was employed by Heel for biometric services. All authors had access to all study data and reviewed and approved the final version of the manuscript for publication and agreed with its contents and submission.

Author contributions

M.W. conceived the experiments. **J.V.M.** conducted the experiments. **S.A.**, **H.J.**, and **M.K.** preprocessed resting-state EEG data. **G.S.**, and **M.K.** implemented VIGALL algorithm on EEG data. **T.C.**, **S.A.**, **H.J.**, and **M.K.** analyzed the results. **T.C.**, **S.A.**, **L.H.**, and **H.J.** wrote the manuscript and all authors reviewed interim drafts and final version of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2021.09.002.

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Supplementary Table S1. Qualitative and quantitative composition of Neurexan®.

Active substance (homeopathic denomination)	**Used plant part / Starting material GHP method**	**Potency (Calculated potency corresponding to final dilution)**	**Mass per 1 tablet (mg)**	**Amount per daily standard dose (3 tablets)**	**Amount per maximum daily dose (12 tablets)**
Avena sativa Avena sativa L.	Fresh, aerial parts harvested during flowering season GHP method 1a dry residue ≥ 2 %; ǿ = ½ part pressed juice	2	0.6	1.8 mg D2 = 180 µg D1 = 36 µg mother tincture (contains 18 µg expressed juice)	7.2 mg D2 = 720 µg D1 = 144 µg mother tincture (contains 72 µg expressed juice)
Coffea arabica (Coffea) Coffea arabica L.	Ripe, dried, unrosted seeds deprived from the exocarp GHP method 4a; ǿ ≥ 0.1% caffeine	12	0.6	1.8 mg D12 = 1.8×10^{-11} mg D1 = mother tincture with at least 1.8×10^{-14} mg alkaloids	7.2 mg D12 = 7.2×10^{-11} mg D1 = mother tincture with at least 7.2×10^{-14} mg alkaloids
Passiflora incarnata Passiflora incarnata L.	Fresh aerial parts GHP method 3a dry residue ≥ 1.6 %	2	0.6	1.8 mg D2 = 180 µg D1 = 54 µg mother tincture	7.2 mg D2 = 720 µg D1 = 216 µg mother tincture
Zincum isovalerianicum (Zincum valerianicum) Zinc oxide + isovalerianic acid $Zn(C_5H_9O_2)_2$ x	Zinc isovalerianate (in German: Baldriansaures Zink) GHP method 5a D2 = 0.93 – 1.08 % substance triturations HAB 6 D1 = 9.3 – 10.8 % substance	4	0.6	1.8 mg D4 = 18 µg D2 with ca. 0.18 µg substance	7.2 mg D4 = 72 µg D2 with ca. 0.72 µg substance

$2H_2O$					

Abbreviations: D = decimal potency, GHP = German Homeopathic Pharmacopoeia, Homöopathisches Arzneibuch (HAB)

frontiers in Neuroscience

ORIGINAL RESEARCH
published: 02 July 2020
doi: 10.3389/fnins.2020.00645

Heart Rate Variability as an Index of Differential Brain Dynamics at Rest and After Acute Stress Induction

Tara Chand[1,2,3,4], Meng Li[2,4], Hamidreza Jamalabadi[1,4], Gerd Wagner[2], Anton Lord[4,5], Sarah Alizadeh[1,4], Lena V. Danyeli[2,4,6], Luisa Herrmann[1,2,4], Martin Walter[1,2,3,4,6] and Zumrut D. Sen[1,2,4]**

[1] Department of Psychiatry and Psychotherapy, University of Tübingen, Tübingen, Germany, [2] Department of Psychiatry and Psychotherapy, Jena University Hospital, Jena, Germany, [3] Max Planck Institute for Biological Cybernetics, Tübingen, Germany, [4] Clinical Affective Neuroimaging Laboratory (CANLAB), Magdeburg, Germany, [5] QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia, [6] Leibniz Institute for Neurobiology, Magdeburg, Germany

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Edited by:
Luca Faes,
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Institut für Innovative Gesundheitstechnologien (IGHT), Germany

***Correspondence:**
Martin Walter
Martin.Walter@med.uni-jena.de;
martin.walter@med.ovgu.de
Zumrut D. Sen
zumrutduygusen@gmail.com

Specialty section:
This article was submitted to Autonomic Neuroscience, a section of the journal Frontiers in Neuroscience

Received: 13 January 2020
Accepted: 25 May 2020
Published: 02 July 2020

Citation:
Chand T, Li M, Jamalabadi H, Wagner G, Lord A, Alizadeh S, Danyeli LV, Herrmann L, Walter M and Sen ZD (2020) Heart Rate Variability as an Index of Differential Brain Dynamics at Rest and After Acute Stress Induction. Front. Neurosci. 14:645. doi: 10.3389/fnins.2020.00645

The brain continuously receives input from the internal and external environment. Using this information, the brain exerts its influence on both itself and the body to facilitate an appropriate response. The dynamic interplay between the brain and the heart and how external conditions modulate this relationship deserves attention. In high-stress situations, synchrony between various brain regions such as the prefrontal cortex and the heart may alter. This flexibility is believed to facilitate transitions between functional states related to cognitive, emotional, and especially autonomic activity. This study examined the dynamic temporal functional association of heart rate variability (HRV) with the interaction between three main canonical brain networks in 38 healthy male subjects at rest and directly after a psychosocial stress task. A sliding window approach was used to estimate the functional connectivity (FC) among the salience network (SN), central executive network (CEN), and default mode network (DMN) in 60-s windows on time series of blood-oxygen-level dependent (BOLD) signal. FC between brain networks was calculated by Pearson correlation. A multilevel linear mixed model was conducted to examine the window-by-window association between the root mean square of successive differences between normal heartbeats (RMSSD) and FC of network-pairs across sessions. Our findings showed that the minute-by-minute correlation between the FC and RMSSD was significantly stronger between DMN and CEN than for SN and CEN in the baseline session [$b = 4.36$, $t(5025) = 3.20$, $p = 0.006$]. Additionally, this differential relationship between network pairs and RMSSD disappeared after the stress task; FC between DMN and CEN showed a weaker correlation with RMSSD in comparison to baseline [$b = -3.35$, $t(5025) = -3.47$, $p = 0.006$]. These results suggest a dynamic functional interplay between HRV and the functional association between brain networks that varies depending on the needs created by changing conditions.

Keywords: heart rate variability, resting-state fMRI, dynamic functional connectivity, heart-brain interaction, stress

INTRODUCTION

The body and the brain are interconnected by dynamic structural and functional networks. These networks provide multi-level interactions and allow conscious and subconscious reactions to constantly changing environmental conditions (McCraty et al., 2009; Smith et al., 2017). Network physiology proposes a new framework to understand the coordination and information integration across different organ systems that give rise to various physiologic states at organism level (Ivanov et al., 2016). Interactions between the brain regions, organs, and organ systems vary dynamically, allowing the same network structure or subsections of the network to be associated with many physiological and psychological states (Honey et al., 2007; Bashan et al., 2012; Bartsch et al., 2015). Recent research on the dynamic temporal interaction between the heart and the brain enriched our understanding beyond the known anatomical connections and physiological regulations (Bashan et al., 2012; Chang et al., 2013; Bartsch et al., 2015; Faes et al., 2016; Young et al., 2017).

Interaction between the cardiovascular system and central nervous system (CNS) is facilitated through reflex arches and the modulatory action of the cortical networks upon them. The central autonomic network (CAN; Benarroch, 1993) refers to a functional unit of brain areas that modulate the autonomic activity depending on the organism's current and expected needs. This network includes brainstem regions, such as dorsal vagal motor nucleus or nucleus of the solitary tract, higher subcortical regions (i.e., hypothalamus and amygdala), and cortical regions [i.e., anterior cingulate cortex (ACC), insula, and medial prefrontal cortex (mPFC)], and modulates the balance between the activity of sympathetic and parasympathetic systems (Benarroch, 1993; Beissner et al., 2013; Dampney, 2015; Shoemaker and Goswami, 2015). Concurrent analyses of heart rate (HR) or heart rate variability (HRV) and blood-oxygen-level dependent (BOLD) signal at rest or during an emotional, cognitive, or motor task indicated that some additional cortical regions, such as dorsolateral PFC (dlPFC), exert influence on HRV in coordination with CAN, although they are not closely connected to the autonomic centers in the brain stem (Napadow et al., 2008; Smith et al., 2014; Young et al., 2017). HRV corresponds to the variation in the time interval between two successive R waves and has been associated with the various cognitive (Hansen et al., 2009; Dalise et al., 2020) and affective functions (Melzig et al., 2009; Miller et al., 2019). Thayer and Lane (2000) put forward the neurovisceral integration (NVI) model centered on CAN regions and provided a framework for understanding the integration of cognitive, affective, and autonomic information. HRV was proposed as an index of the degree to which flexible and adaptive interaction between the human organism and the environment can be achieved (Thayer and Lane, 2000, 2009; Thayer and Brosschot, 2005; Thayer et al., 2012).

Smith et al. (2017) extended the NVI model and described the roles in efferent and afferent information processing of each brain region constituting the CAN. The lower levels of hierarchy correspond to the information processing at the level of the brainstem nuclei and subcortical regions that integrate upcoming information from different bodily sources (Smith et al., 2017). On the other hand, the core neural networks, such as the Default Mode Network (DMN) and the Salience Network (SN), constitute the higher levels of hierarchy, where information in terms of exteroception, interoception, and memory is integrated by taking into consideration not only the present, but also the expected future metabolic needs, which are related to long-term goals. The Central Executive Network (CEN) processes goal-relevant information within a circuit of highly connected hub regions that constitute SN and DMN (Dehaene, 2014; Barrett, 2016; Smith et al., 2017). The synchronous activity of these networks may provide a "global workspace" that allows the emergence of conscious representations that are significant to the goals or overall state of the organism (Zylberberg et al., 2010, 2011; Dehaene and Sigman, 2012). These three core brain networks, namely CEN, SN, and DMN, have been identified by functional connectivity (FC) analyses predominantly at resting state fMRI, while subjects lie in the scanner and are not asked to engage in any particular task. Since the ascending inputs from the visceral organs continuously reach numerous cortical and subcortical regions, many researchers claim that the visceral signals are the continuous internal stimuli that contribute and shape the spontaneous brain activity and intrinsic brain-networks (Azzalini et al., 2019; Kim et al., 2019).

If HRV is an index of the flexible interaction between the heart and the brain, the relationship between HRV and FC patterns in the brain networks should vary across different psychological and physiological states. Stress is a perturbed state at the whole-body level induced by extrinsic or intrinsic stimuli (Oken et al., 2015). Stress induction activates limbic regions such as amygdala, which is also under influence of cortical regions such as vmPFC; consequently, hypothalamic-pituitary-adrenal axis and sympathetic nervous system are activated, leading to increased cortisol levels and heart rate (Tafet and Nemeroff, 2016). HRV as measured by the root mean square of successive differences between normal heartbeats (RMSSD) or high frequency (HF) power are suppressed as a reaction to acute stress induction (Kim et al., 2018; Castaldo et al., 2019). Three dominant brain networks, namely DMN, CEN, and SN, are known to be modulated by acute stress (Hermans et al., 2011; Vaisvaser et al., 2013; Maron-Katz et al., 2016; van Oort et al., 2017). For example, during acute stress induction by affective stimuli, the activation and functional connectivity of SN as well as the activation in DMN regions increase, while the activation in CEN remains unchanged (van Oort et al., 2017). On the other hand, less consistent findings were reported during acute stress induction by psychosocial stress tasks, while the role of brain regions being part of CEN, SN, and DMN is consistently reported during stress induction (van Oort et al., 2017). Previous rs-fMRI studies indicated a carry-over effect of acute psychosocial stress induction on these three intrinsic brain-networks (Vaisvaser et al., 2013; Maron-Katz et al., 2016). An increased functional connectivity between brain regions of the SN and DMN was associated with the subjective stress levels (Vaisvaser et al., 2013; Quaedflieg et al., 2015; Maron-Katz et al., 2016). However, whether the association of HRV with the FC between these three networks changes before and after stress induction remains to be tested.

More recent views of resting-state FC (rsFC) integrate both static and dynamic components: the static component represents stable dimensions of overall FC during the whole fMRI session, and the dynamic component represents the processes by which networks and subnetworks unite and dissolve over time (Chang and Glover, 2010; Handwerker et al., 2012; Hutchison et al., 2013; Kaiser et al., 2016). Indeed, dynamic FC (dFC) was shown to co-fluctuate with HRV and arousal (Chang et al., 2013; Young et al., 2017). By using a sliding window approach to calculate FC maps and HRV, Chang et al. (2013) showed that FC between dorsal ACC and precuneus was significantly associated with the HRV. However, the association of HRV with the dynamic and static FC between the three core brain networks remains to be elucidated.

Here, we investigated the temporal association between RMSSD and FC between the three core brain networks – DMN, SN, and CEN – at two different resting-state scan sessions: baseline and just after a psychosocial stress task. In this study, we hypothesized that RMSSD correlates with the FC between the three network pairs (DMN-SN, DMN-CEN, and SN-CEN) at rest and these patterns are altered by acute stress. Minute-by-minute associations were assessed by the multilevel analysis instead of the common summary-statistics model (Chang et al., 2013; Vaisvaser et al., 2013; Young et al., 2017), in which first-level (e.g., subject or time window) data are aggregated on group level and the variance of first-level data cannot be taken into account. Moreover, this is the first study to examine how the functional association of HRV with FC between the core brain networks change after acute stress induction.

MATERIALS AND METHODS

Sample

This study was part of a randomized, placebo-controlled, double-blind, two-period crossover clinical trial (ClinicalTrials.gov Identifier: NCT02602275). The Ethics Committee of the Medical Faculty of the University of Magdeburg approved the experimental protocol of the study and the study was conducted in accordance with the Declaration of Helsinki (World Medical Association, 2002). Participants provided written informed consent prior to participation and received financial compensation for their participation. Participants were screened for MR compatibility and medical and psychiatric examination, including a Structured Clinical Interview for DSM-IV Axis I, (SCID) was performed. During the screening visit, the stress level was assessed by means of the Perceived Stress Scale (PSS) and Trier Inventory for Chronic Stress – Screening Scale for Chronic Stress (TICS-SSCS). Subjects with low (PSS score = 9, TICS-SSCS score < 9) and high (TICS-SSCS score > 36) levels of chronic stress were not included in the study to ensure subject's susceptibility to the stress and to avoid a ceiling effect of the stress sensitivity. Subjects were excluded if they were diagnosed with any psychiatric or serious somatic disease or were not suitable for MRI scanning.

A group of 40 healthy male subjects aged 31–59 years was enrolled in the study. Since males and females showed distinct stress responses modulated by the difference in hormonal levels caused partly by menstrual cycle (Goldstein et al., 2010; Saladin et al., 2015), only male participants were recruited for this study to reduce the variability within stress responses. The sequence of placebo or active compound was randomized. After randomization, one subject was excluded because of an incidental finding during the baseline MR measurement; therefore, 39 subjects (age = 43.7 ± 9.8) were included in this study. Nineteen participants received a placebo and the remaining 20 participants received the *verum* on the first measurement day. On the second measurement day, after a wash out period (7–35 days after the first measurement day), treatment was crossed-over.

Study Design

On both measurement days, Days 1 and 2, after an anatomy scan and acquisition of 12-min baseline rs-fMRI (RS0), participants received either placebo or *verum* [a herbal medicinal product, Neurexan (Nx4)]. Subsequently, they performed two attention tasks with simultaneous EEG acquisition outside the scanner before entering another fMRI session. During that session, participants underwent a psychosocial stress paradigm, which was preceded by two emotion-related fMRI tasks reported in separate publications. A 12 min resting-state sequence was acquired before the fMRI tasks (RS1) as well as immediately after the psychosocial stress induction (RS2) (see **Figure 1**). Here in the current study, only RS0 (baseline) and RS2 (after stress induction) scans of the day of placebo intake were analyzed. RS1 scans were not included in the main analysis, but findings obtained from the analysis of all three scanning time points are reported in the **Supplementary Results**. The photoplethysmography (PPG) signal was continuously monitored during all scans. The PPG sensor was attached to the proximal phalanx of the left index finger.

To induce psychosocial stress, we used a shortened version of the ScanSTRESS task inside the scanner, which was previously shown to induce stress-related changes in the brain, including changes in physiological measures and hormone levels (Streit et al., 2014, 2017; Dimitrov et al., 2018). During the task, participants performed demanding arithmetic calculations and mental rotations under time pressure and with feedback regarding correctness and speed from a jury panel shown live on the screen (stress blocks). As a control condition, the questions of both types were easy and there was no time pressure or evaluative feedback (control blocks). In total, each block of 40 s (shortened version) was presented four times, with stress and control blocks on an alternating basis. In the middle of the experiment, participants received negative verbal feedback from the panel to increase the stress level. The detailed description of the task can be found in Streit et al. (2014).

The level of anxiety and nervousness was assessed using the State-Trait Anxiety Inventory (STAI-X1) and a continuous visual analog scale for nervousness (VAS-Nerv) at baseline as well as before and after stress induction. Saliva samples for alpha-amylase and cortisol measurements were collected at eight-time points before and after the stress task via the saliva collection device Salivette® (Sarstedt, Germany) in addition to morning cortisol samples. Salivary cortisol and alpha-amylase levels

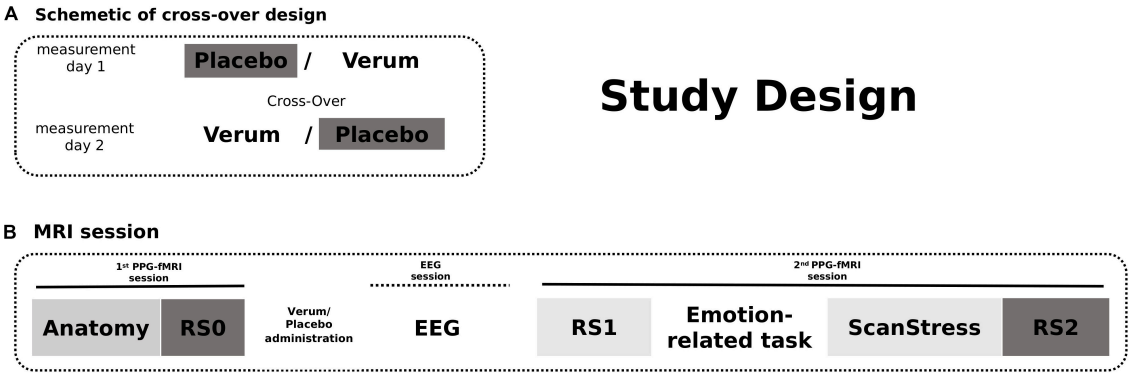

FIGURE 1 | The study design. **(A)** Participants received either Verum or Placebo on two measurement days. The treatment was cross-overed. **(B)** On both measurement days, fMRI scans were acquired in two sessions. The first session began with an anatomical scan followed by a baseline resting-state measurement. After intake of the Placebo/Verum treatment, EEG paradigms were performed outside the scanner. The second MRI session included the shortened ScanStress task measurement following two other task measurements as well as two resting-state measurements before and after the tasks, respectively. In this publication, only the analyses of RS0 and RS2 fMRI scans on the day of placebo intake were reported.

were analyzed using commercial enzyme-linked immunosorbent assays according to the manufacturer's instructions.

MRI Acquisition

All data (structural and functional MRI) were acquired on a Philips 3T scanner in Magdeburg. First structural T1-weighted images were measured using a TFE sequence with the following parameters: 274 sagittal slices covering the whole brain, flip angle = 8°, 256 × 256 matrix, voxel size 0.7 × 0.7 × 0.7 mm^3. The functional MRI data were acquired using following scanner settings: 34 axial slices covering the whole brain, TR = 2,000 ms, TE = 30 ms, flip angle = 90°, 96 × 94 matrix, field of view = 240 × 40 mm^2, voxel size = 2.5 × 2.5 × 3 mm^3. For the resting-state sessions (before placebo administration, before and after the tasks), 355 volumes of T2*- weighted echo-planar images (EPIs) were acquired for each session with the same parameters. All subjects were instructed to keep their eyes closed, to not think of anything specific, and to not fall asleep during the resting-state measurements.

fMRI Preprocessing

Rs-fMRI-data were analyzed in MATLAB 2017 (The Mathworks Inc., Natick, MA, United States) using the SPM12 (Wellcome Department of Imaging Neuroscience, London, United Kingdom)[1] and CONN toolboxes (Whitfield-Gabrieli and Nieto-Castanon, 2012). Preprocessing of the rs-fMRI data was performed using the adapted preprocessing pipeline in CONN. The pipeline includes motion correction (realignment and unwarping), slice-timing correction, automatic detection of artifactual scans (ART-based scrubbing, Mazaika et al., 2005), and normalization to MNI space. The CONN toolbox-featured intermediate scrubbing parameters were used to compute head motion in each session of each subject. In the next step, we evaluated the sessions in which head motion exceeded the threshold in more than 25% of volumes. This head motion criterion resulted in the exclusion of one subject's RS0 and one subject's RS2 session. Single-subject linear regression analyses were performed to remove effects of head motion (12 total motion covariates: six motion parameters plus temporal derivatives), physiological artifacts [10 total eigenvariates based on the anatomical component-based noise correction method (aCompCor, Chai et al., 2012): five each from eroded white matter (WM) and cerebrospinal fluid (CSF) masks], and artifactual scans in each subject during denoising in CONN. The resulting residual BOLD time series were band-pass filtered (0.01–0.25 Hz) and spatially smoothed with a 6 mm Full-Width at Half-Maximum (FWHM) Gaussian kernel. Finally, each time series was normalized to zero mean and unit variance (z-value), to reduce variance of non-neural origin (Huang et al., 2018). One single subject was not preprocessed due to the corruption of the data, therefore, in total 38 subjects (37 for each session) were included in the analysis.

FC Between Network-Pairs

Key regions of three canonical brain networks were identified following prior literature (Uddin et al., 2011). As shown in **Table 1**, the resulting 10 region of interests (ROIs) were constructed by drawing spheres of 5 mm radius around the following key nodes: the DMN (vmPFC and PCC), SN [bilateral fronto-insular cortex (FIC) and rostral ACC (rACC)], and CEN (bilateral dlPFC and PCC) (**Figure 2A**).

A sliding-window approach was used to calculate the FC between network-pairs (**Figure 2B**). The mean time course of each brain network was extracted for the windows of 60 s with a 50% overlap. The total number of windows for each session was 23. Pearson-correlation was calculated for the FC between network-pairs.

Physiological Recordings and HRV Calculation

A finger PPG signal with a sampling rate of 500 Hz was acquired using the scanner's built-in equipment concurrently with fMRI.

[1] http://www.fil.ion.ucl.ac.uk

TABLE 1 | Composition of the canonical networks.

Network	Region	MNI coordinates (mm)
SN	rFIC	39, 23, −4
	lFIC	−34, 20, −8
	ACC	6, 24, 32
CEN	rDLPFC	46, 20, 44
	lDLPFC	−46, 20, 44
	rPPC	52, −52, 50
	lPPC	−40, −56, 44
DMN	vmPFC	−2, 38, −12
	PCC	−6, −44, 34

SN, salience network; DMN, default mode network; CEN, central executive network; rFIC, right fronto-insular cortex; lFIC, left fronto-insular cortex; rDLPFC, right dorsal lateral prefrontal cortex; lDLPFC, left dorsal lateral prefrontal cortex; rPPC, right posterior parietal cortex; lPPC, left posterior parietal cortex; VMPFC, ventrolateral prefrontal cortex; PCC, posterior cingulate cortex.

During data acquisition, local maxima of PPG signal were automatically detected and timestamps of PPG peaks (P-peaks) were recorded by the MR scanner. The inter-beat intervals (IBIs) were calculated by extracting the time interval between subsequent P-peaks. Prior to the calculation of the inter-beat intervals, the quality of the peaks was manually inspected using an in-house MATLAB script by overlapping scanner detected P-peaks timestamps over PPG signal; missing peaks were added manually. The intermittent errors in P-peaks due to the ectopic beats or movement artifact were identified using percentage filter (IBIs increase or decrease of more than 30% compared to the previous interval) and subsequently interpolated (Salo et al., 2001; Ramshur, 2010; Peltola, 2012; Choi and Shin, 2018). RMSSD is the root mean square of the successive difference in adjacent IBIs, which measures the short-term variations of the IBI signal (Task Force of ESC and NASPE, 1996; Shaffer and Ginsberg, 2017). RMSSD was calculated for each individual sliding window of 60 s (**Figure 2C**).

Statistical Analyses

The data of the current study was comprised of two resting-state sessions and 23 sliding window estimations for each subject. To assess dynamical temporal associations, the multilevel analysis was preferred over a summary-statistics model. A multilevel mixed linear model (**Figure 2D**) was build using the fitlme command of Statistics Toolbox in MATLAB. Window-by-window FC between network-pairs was taken as the dependent variable, while session (RS0 and RS2), network-pair (DMN-CEN, DMN-SN, and SN-CEN) and window-by-window HRV were added as regressors. The HRV values were centered according to the subject level mean HRV values for each session separately (Finch, n.d.). Fixed effects were calculated for the regressors and their interaction terms. The random term was defined as network-pairs nested under sessions and subjects. The random intercept model with a diagonal covariance matrix was chosen based on the Akaike Information Criterion (AIC) fit index. Parameters were estimated using the restricted maximum likelihood estimation (REML) method, which has been proven to be more accurate than maximum likelihood estimation (MLE) for estimating variance parameters (Kreft and De Leeuw, 1998; Finch, n.d.). The normality of residuals and homoscedasticity were investigated by plotting the normal probability of residuals and of residuals vs. fitted values (**Supplementary Figure S1**). Bonferroni adjustment was used for multiple comparison correction in *post-hoc* analyses (in total six pair-wise comparisons across the regression coefficients of each network-pair in each session). Mean HRV and mean HR in each session were also added separately to the above-specified model as second-level variables. To investigate the effect of acute stress induction on the temporal association of dFC between network-pairs, the parameters related to stress response, such as the difference between saliva alpha-amylase and cortisol levels before and after stress induction, and the difference between HR and ratings of VAS-Nerv, were added to the model on subject level. Age and period of placebo intake (Day 1 or Day 2) were also controlled for.

The effect of acute stress induction on mean HR was examined by one-way rmANOVA (Session: RS0, RS1, ScanSTRESS task and RS2). Greenhouse-Geisser was used to adjust the degrees of freedom for the averaged tests of significance. Additionally, the carry-over effect of stress induction on subjective and physiological measures was analyzed by conducting paired sample *t*-tests between RS0 and RS2, using mean HR and mean HRV during scans, saliva cortisol, and alpha-amylase levels, and scores of STAI-XI and VAS-Nerv at the time of each scanning time point as dependent variables. The statistical threshold was set to $\alpha = 0.05$.

RESULTS

The Carry-Over Effect of Acute Stress Induction on Subjective and Physiological Measures

To investigate the carry-over effect of acute stress induction on subjective and physiological measures, paired sample *t*-tests were performed across RS0 and RS2. As depicted in **Figure 3**, a significant increase in nervouseness [$t(37) = -2.44$, $p = 0.019$] and HR [$t(37) = -5.78$, $p < 0.001$] were observed. No significant change was observed on the level of salivary cortisol and alpha-amylase, and mean RMSSD.

The Dynamic Temporal Association of HRV With FC Between Network-Pairs

Analysis using a multilevel linear mixed model was conducted by taking window-by-window FC (dFC) between network-pairs as the dependent variable and window-by-window HRV, network-pairs and session as regressors, while subject level random terms for each session were introduced. HRV was significantly associated with dFC between DMN-CEN [$b = 3.35$, $t(5025) = 3.47$, $p < 0.006$ (Bonferroni corrected)] in the baseline condition (**Table 2**). As depicted in **Figure 4A**, this association was significantly higher than that between SN-CEN dFC and HRV [$b = -4.36$, $t(5025) = -3.20$, $p < 0.006$ (Bonferroni corrected)] at baseline (**Table 3**).

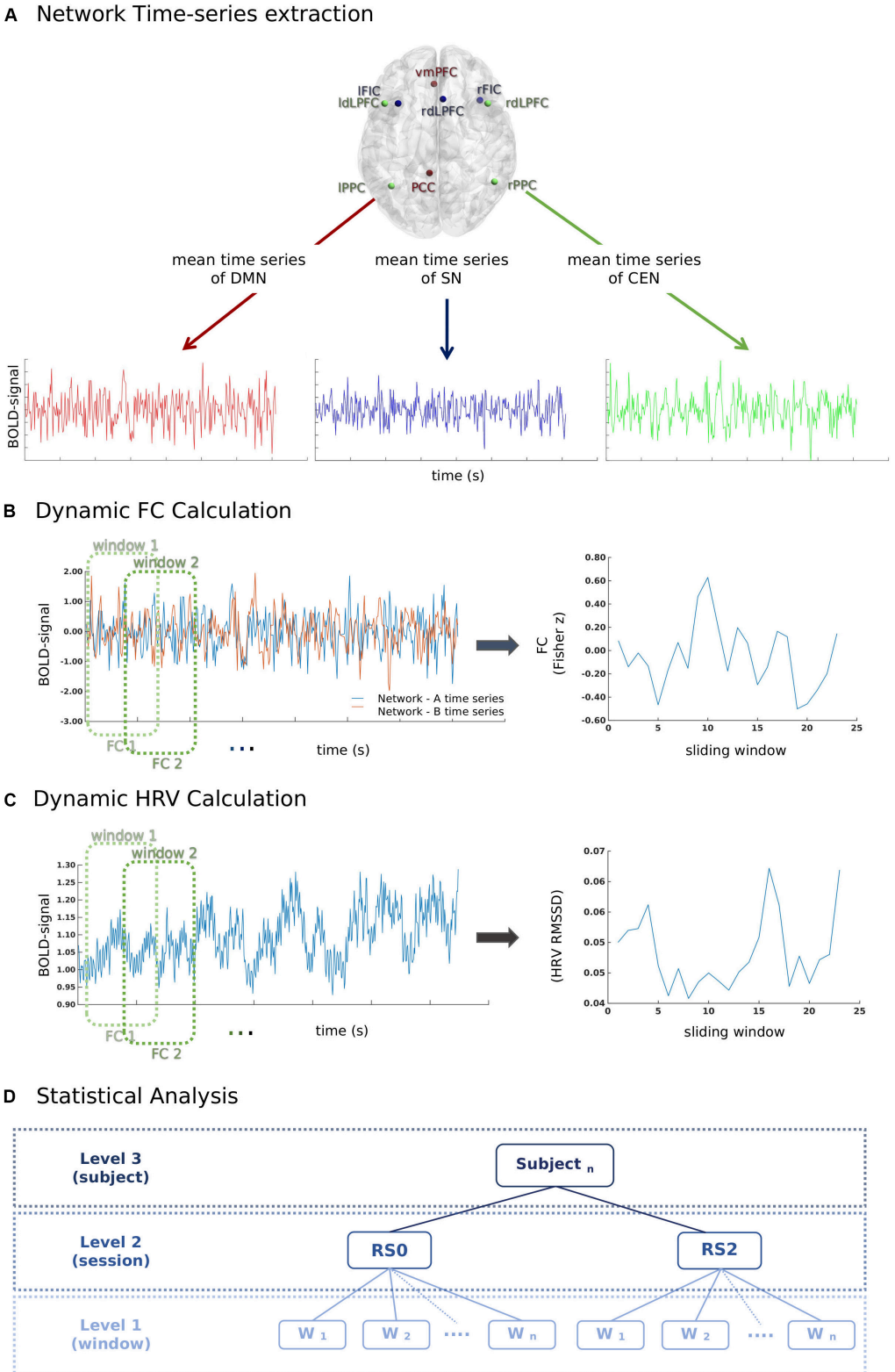

FIGURE 2 | A schematic illustration of the methods used for the calculation of the temporal relationship between heart rate variability (HRV) and functional connectivity (FC) between network-pairs. **(A)** First, time series of mean BOLD signals in each network were extracted for each window (60 s) according to the sliding-window analysis approach. **(B)** Pearson-correlation was performed for the calculation of FC between network-pairs. **(C)** RMSSD was calculated from the inter-beat interval time series for each window (60 s) according to the sliding-window analysis approach. **(D)** The relationship between window-by-window RMSSD and FC between network-pairs was analyzed using a multilevel linear mixed model.

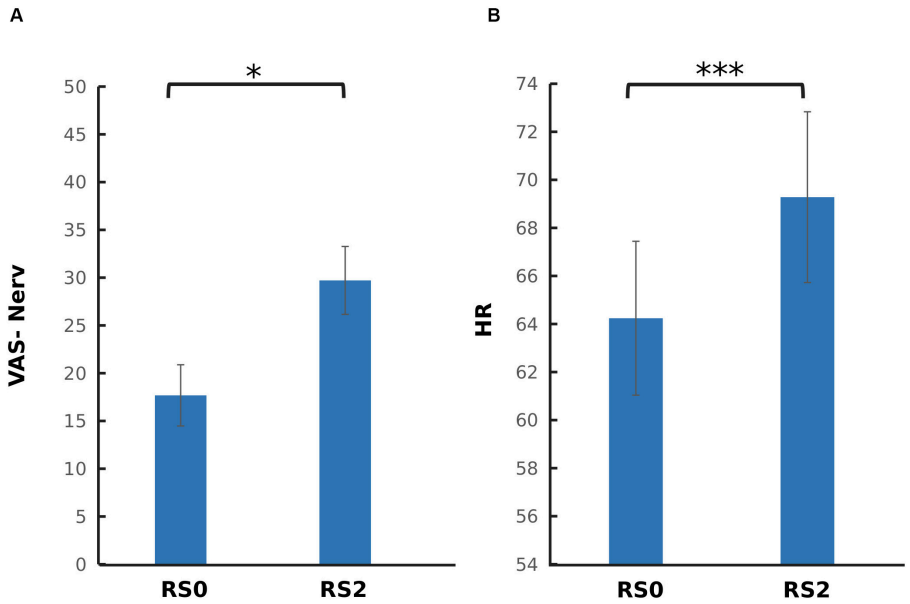

FIGURE 3 | Comparison of psychophysiological parameters between the two sessions. **(A)** Visual analog scale for nervousness (VAS-Nerv) **(B)** Mean heart rate (HR). After the acute stress induction, a significant increase in the level of nervousness, and mean HR was found. The error bars represent standard error. *$p < 0.05$; ***$p < 0.001$.

TABLE 2 | Correlation of RMSSD with dFC between network-pairs for each session (RS0 and RS2).

Session	FC	Estimate	SE	T	Adjusted *P*-value
RS0	DMN-SN	1.90	0.96	1.97	0.288
	DMN-CEN	3.35	0.96	3.47	**0.003****
	SN-CEN	−1.01	0.96	−1.05	1.000
RS2	DMN-SN	0.36	0.94	0.38	1.000
	DMN-CEN	−0.93	0.94	−0.99	1.914
	SN-CEN	1.53	0.93	1.64	0.600

*RMSSD = Root mean square of the successive difference in adjacent IBIs; dFC, dynamic functional connectivity; RS0, Baseline session; RS2, After stress induction; SN, Salience network; DMN, Default mode network; CEN, Central executive network; Adjusted p-value, Bonferroni corrected p-values. Bold font represents significant results; ** represents p < 0.01.*

The relationship between HRV and DMN-CEN-dFC significantly decreased after acute stress induction [$b = -3.35$, $t(5025) = -3.47$, $p < 0.006$ (Bonferroni corrected)] (**Figure 4B** and **Table 4**) and was not significant at the RS2.

These effects were also observed after controlling for age and the period of placebo intake, as well as mean HR and mean HRV during each session. Moreover, additional analyses including the difference between saliva alpha-amylase and cortisol levels, and the difference between ratings of VAS-Nerv parameters before and after stress induction did not alter the above-mentioned findings.

DISCUSSION

In this study, we examined the association of RMSSD with dFC between brain networks at baseline and right after a psychosocial stress task in a sample of 38 healthy male subjects. By using multilevel regression analysis, we demonstrated that the minute-by-minute association between the dFC and RMSSD was significantly stronger for the interaction between DMN and CEN than between SN and CEN in the baseline session. This difference between dFC of network-pairs in terms of their association with RMSSD disappeared after the stress task. Furthermore, dFC between DMN and CEN showed a weaker correlation with RMSSD after the stress task in comparison to baseline. Moreover, these findings were replicated with one additional resting-state scan (RS1), which was acquired after placebo intake and before the stress induction (**Supplementary Results**). These results indicate a dynamic functional relationship between HRV and brain networks that varies depending on external conditions. To the authors' knowledge, this is the first report suggesting the pattern of functional associations between HRV and the interaction between different brain networks during resting state, and how this changes immediately after acute stress induction.

The significant correlation between RMSSD and dFC of DMN and CEN in the baseline session suggests that the CEN nodes, such as dlPFC and posterior parietal cortex (PPC), are functionally related to the rhythmic activity of the heart, even though they are not part of the original definition of CAN (Benarroch, 1993). The stronger functional association of RMSSD with the interaction between CEN and DMN compared with CEN and SN during the baseline session is in line with the previously mentioned extended NVI model, in which the role of CEN in vagal control was proposed (Smith et al., 2017). This model describes that DMN plays a role in the conceptualization of the visceral and somatic information, thereby representing the conceptual significance of the overall situation of the organism in a given context, while SN plays a regulatory role at the level

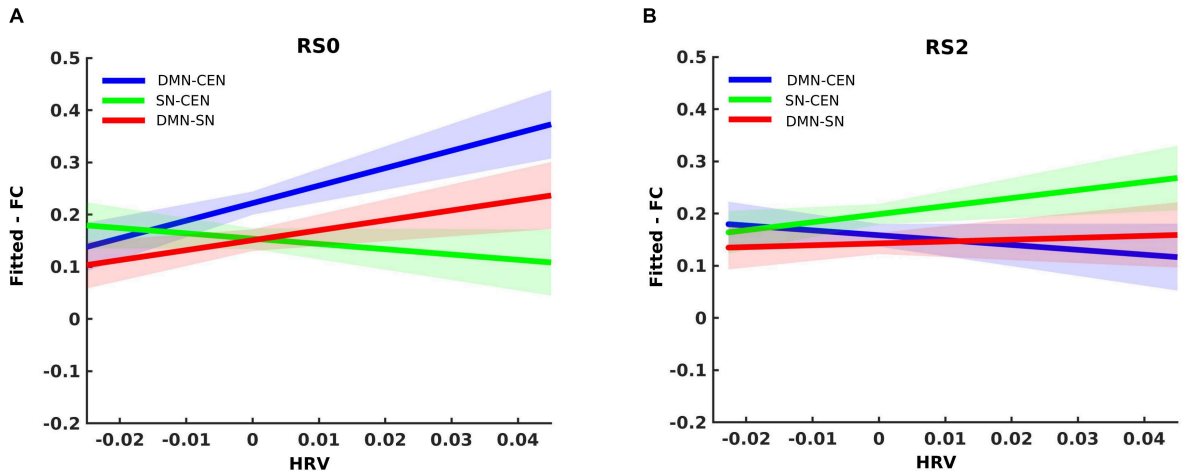

FIGURE 4 | Differential temporal association between heart rate variability (HRV) and functional connectivity (FC) between network-pairs across sessions (RS0 and RS2). **(A)** The multilevel linear mixed effect model showed a significant correlation between HRV and FC between DMN-SN, and DMN-CEN during baseline (RS0). The strength of the association between HRV and FC was significantly stronger for DMN-CEN than for SN-CEN during baseline session (RS0). **(B)** The correlation between HRV and FC between DMN-CEN was significantly weaker during the second session (RS2) in comparison to baseline. RS0, Baseline Session; RS2, After stress induction; SN, Salience Network; DMN, Default Node Network; CEN, Central Executive Network. Shaded areas indicate standard error.

TABLE 3 | Within-session comparisons of correlation strengths between RMSSD and network-pairs dFC.

Session	Reference NP	Target NP	Estimate	SE	T	Adjusted *P*-value
RS0	DMN-SN	SN-CEN	−2.92	1.36	−2.13	0.192
	DMN-CEN	DMN-SN	−1.45	1.36	−1.06	1.000
	DMN-CEN	SN-CEN	−4.36	1.36	−3.20	**0.006****
RS2	DMN-SN	SN-CEN	1.18	1.32	0.89	1.000
	DMN-CEN	DMN-SN	1.28	1.32	0.97	1.000
	DMN-CEN	SN-CEN	2.47	1.32	1.87	0.372

*RMSSD, Root mean square of the successive difference in adjacent IBIs; dFC, dynamic functional connectivity; NP, Network-pair; RS0, Baseline session; RS2, After stress induction; SN, Salience network; DMN, Default mode network; CEN, Central executive network; Adjusted P-value, Bonferroni corrected p-values. Bold font represents significant results; **p < 0.01.*

TABLE 4 | Between-session comparisons of correlation strengths between RMSSD and network-pairs dFC.

NP	Reference session	Target session	Estimate	SE	T	Adjusted *P*-value
DMN-SN	RS0	RS2	−1.54	1.34	−1.23	1.000
DMN-CEN	RS0	RS2	−4.28	1.34	−3.18	**0.006****
SN-CEN	RS0	RS2	2.55	1.34	1.90	0.342

*RMSSD, Root mean square of the successive difference in adjacent IBIs; dFC, dynamic functional connectivity; NP, Network-pair; RS0, Baseline session; RS2, After stress induction; SN, Salience network; DMN, Default mode network; CEN, Central executive network; Adjusted P-value, Bonferroni corrected p-values. Bold font represents significant results; **p < 0.01.*

of perception (Barrett and Satpute, 2013; Smith et al., 2017). The synchronous activity of SN and DMN with CEN enables maintenance and further processing of relevant information (Dehaene, 2014; Barrett, 2016; Smith et al., 2017). Activation and FC of DMN regions are associated with spontaneous and self-generated thoughts under resting conditions (Raichle et al., 2001; Buckner et al., 2008; Buckner and DiNicola, 2019). On the other hand, SN activation and FC are induced in response to salient and affective stimuli and play a role in the allocation of attention (Seeley et al., 2007; Hermans et al., 2011, 2014; Menon, 2011). Therefore, under resting conditions, the dominance of DMN was expected. In this study, the static rsFC between CEN and DMN is stronger than between CEN and SN at baseline (**Supplementary Results**). Presumably, HRV is primarily related to the currently dominant process across brain networks, which is expected to mirror the needs of the body, while on the other hand, HRV also reflects the processes in the brain invoked in the frame of its relation to the body and environment (McCraty and Childre, 2010).

Acute stress induction can induce a reorganization of FC between brain networks to support a hypervigilant state (Hermans et al., 2014). Since the stress response continues directly after stress exposure (van Marle et al., 2010), a carry-over effect of the stress task was expected during the following resting-state scan. Indeed, even the effects of prior cognitive tasks are carried over to the following post-task resting-state brain activity (Albert et al., 2009; Lewis et al., 2009; Hartzell et al., 2015). In this study, the association between HRV and dFC between CEN and DMN was decreased directly after acute stress induction when compared with the baseline session. An increase in FC and activation of SN regions was reported after acute stress induction, while DMN FC was decreased (van Marle et al., 2010; Vaisvaser et al., 2013; Quaedflieg et al., 2015; van Oort et al., 2017). The static rsFC findings of this study showed that rsFC between DMN-CEN was reduced after acute stress induction, which might explain the shift in the association of RMSSD with FC between network-pair. Of note, the carry-over effect of stress induction was observed regardless of the inclusion of mean values of HR,

HRV, or nervousness ratings during each session. Participants reacted to the acute stress induction with an increase in HR during the task, which also did not explain the carry-over effect of stress on the association between HRV and interaction between intrinsic networks.

A point that needs to be taken into consideration is the usage of Pearson correlation coefficient to estimate the strength of FC between network-pairs. The Pearson correlation coefficient does not imply causal relationship and might also reflect an indirect influence by a third region (Wang et al., 2016). Therefore, current findings cannot indicate the direction of an effect between RMSSD and intrinsic brain networks. Even though HRV has been mostly interpreted as an index of the modulatory effect of the CAN on the cardiac activity, the CAN receives continuous information from the cardiovascular system via the cardiac afferent fibers and through blood vessels. Therefore, it is difficult to identify causality for the heart-brain circuit. For this reason, we chose to describe our observations as co-evolution of signals instead of an effect of one on the other. Likewise, we avoided limiting the association of RMSSD and functional association of brain networks in a frame of parasympathetic activity. RMSSD reflects predominantly parasympathetic rather than sympathetic activity (Shaffer and Ginsberg, 2017). However, confidently separating sympathetic and parasympathetic influences on the heart-brain circuit is beyond this study due to the complexity of the heart-brain circuit, which also involves interaction between the branches of the autonomic nervous system (ANS) and the non-linear interplay of all regulatory loops involving the intracardiac nervous system and pacemaker cells in the heart, especially when some of our ROIs are considered, which are not primarily part of the CAN. Moreover, additional analyses with other vagal HRV metrics, such as high-frequency HRV, are required to indicate that the functional association between the core brain regions and RMSSD was driven by parasympathetic activity.

A limitation of the current study is the window size of the 60 s, which might not be the optimal time window to capture the temporal dynamics of the interplay between RMSSD and intrinsic brain networks. While the underlying temporal scale of the reported effects has not been exhaustively assessed, the 60 s window size was used as a compromise between including enough timepoints within a window to provide reliable measures and capturing relatively short-lived effects (Hutchison et al., 2013; Chen et al., 2017). In the current study, the selection of a 60 s window and 50% overlap yielded 23 sliding-window measurements per scan, 30 fMRI timepoints per window for estimating FC and the association between dFC and RMSSD. According to previous findings in the literature, functionally relevant dFC patterns can be isolated from a window size of 60 s (Shirer et al., 2012; Gonzalez-Castillo et al., 2015; Leonardi and Van De Ville, 2015; Liégeois et al., 2017). Furthermore, the calculation of RMSSD from the signal acquired by means of recordings of less than 1 min is still a matter of debate (Laborde et al., 2017). This also corresponds to one of the reasons behind our choice of RMSSD as an HRV index parameter.

Since ECG is more susceptible to radiofrequency artifacts during fMRI scans and the magnetic field within the scanner, the pulse oximetry is generally preferred in MRI settings (Chang et al., 2013; Bellot et al., 2016; Kasper et al., 2017). Even though the utility of the pulse oximeter in calculating HRV by peak detection algorithms was demonstrated (Chang and Glover, 2009; Verstynen and Deshpande, 2011; Nilsson, 2013; Schäfer and Vagedes, 2013; Caballero-Gaudes and Reynolds, 2017), pulse oximeter measurements are delayed due to the pulse transit time. However, in MRI settings pulse oximetry is still more favorable than ECG because of its robustness to artifacts arising from the setting itself.

The HRV signal has a complex structure and involves superimposed oscillations (Ivanov et al., 1999; Pikkujämsä et al., 1999). RMSSD is one of the most commonly used time-domain measures of HRV; however, there are many other options to calculate HRV, such as time-domain, frequency-domain, and non-linear measurements (Shaffer and Ginsberg, 2017). Thus, RMSSD parameter illuminates only a specific and small part of HRV and cannot be considered a full representative of this regulation. Because of the 60 s window size, the calculation of the low-frequency domains is not appropriate from a signal analysis perspective (Task Force of ESC and NASPE, 1996; Shaffer and Ginsberg, 2017). Moreover, the frequency domain and also other time-domain indices are more susceptible to the influence of respiration, which was not controlled for in HRV calculation because neither respiratory rate nor depth was recorded during the scanning. Of note, the validity of ultra-short HRV features (acquisition time less than 5 min) is still under debate (Shaffer and Ginsberg, 2017; Castaldo et al., 2019); however, the use of acquisition times of 60 s and below was also proposed (Salahuddin et al., 2007; Esco and Flatt, 2014; Baek et al., 2015). On the other hand, the conventional recording time of 5 min would not be appropriate to examine the dynamic temporal functional association between the rhythmic activity of the heart and the FC of the core brain networks.

Physiological signals are generally regressed out during pre-processing of fMRI data as respiration and the cardiac cycle can result in neuronal and non-neuronal fluctuations in the BOLD signal due to the systemic changes in arterial CO2 concentrations and blood flow (Birn et al., 2006; Shmueli et al., 2007; Chang and Glover, 2009). In the current study, a CompCor approach was used during pre-processing that extracts multiple nuisance regressors from the voxels within WM and CSF via principal component analysis (Behzadi et al., 2007; Muschelli et al., 2014). A CompCor approach can account for physiological noise (Behzadi et al., 2007) and head motion (Muschelli et al., 2014). As our main interest was the interaction of cardiac activity and inter-network FC, removing the physiological signals from the imaging data further than regressing out the signal intensity from WM and CSF might have resulted in a decrease of the signal of interest (Khalili-Mahani et al., 2013; Zhang et al., 2019). Nevertheless, considering the parallel change between the results of static rsFC between the network-pairs (**Supplementary Results**) and the dynamic association of HRV and dFC between network-pairs, the findings of this study should be interpreted with caution.

These findings, although preliminary, suggest that HRV co-fluctuates with the core brain networks selectively depending on the condition. This combination of findings provides some support for the conceptual premise that the brain and the heart

function in a closely coordinated manner as a part of a bigger psychophysiological system to maintain the homeostatic state of the organism in a constantly changing environment.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Magdeburg. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TC, ZS, SA, LH, HJ, and MW were involved in the development of the study. TC, ML, GW, AL, LD, MW, and ZS were involved in the analysis and interpretation of data. All authors gave approval of the final version and agreed to be accountable for all aspects of the work.

ACKNOWLEDGMENTS

The explored dataset was collected along with EEG data in a clinical trial sponsored by the Heel GmbH, Germany (NCT02602275) in which MW was the PI. fMRI data was provided as a courtesy for the purpose of these analyses, which were not related to the trial objectives. HJ was supported by fortüne grant of Medical Faculty of University of Tübingen (No. 2487-1-0). We thank Nooshin Javaheripour and Vinod Kumar for their contribution regarding the preparation of figures. We would also like to acknowledge and thank all participants in this study. We acknowledge support by Deutsche Forschungsgemeinschaft and Open Access Publishing Fund of the University of Tübingen.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2020.00645/full#supplementary-material

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Conflict of Interest: MW received institutional research support from Heel paid to his institution for this study and from BrainWaveBank and H. Lundbeck A/S outside the submitted work. The University of Tübingen received institutional fees for advisory services by MW from Heel GmbH, Servier Deutschland GmbH, Bayer AG, and Janssen-Cilag GmbH. HJ was supported by the fortüne grant of the Medical Faculty of the University of Tübingen (No. 2487-1-0). TC, ML, LH, LD, and ZS received financial support paid to the instiurion for conference attendance from Heel outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material

1. Normality plots of the multilevel linear mixed model

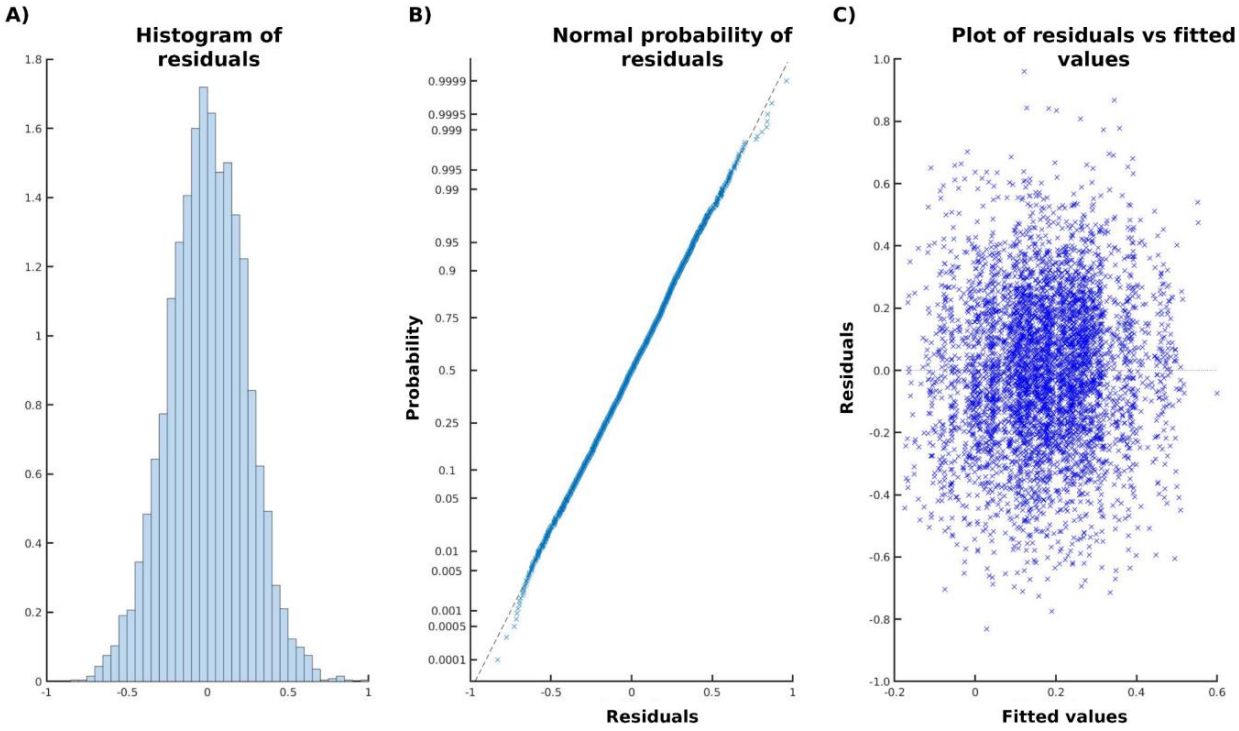


Figure S1: Residual analysis of the multilevel mixed model conducted for the temporal association between heart rate variability and dynamic functional connectivity. **A)** Histogram of residuals. **B)** Test for normal probability of residuals. **C)** Scatterplot of residuals vs. fitted values.

2. Effect of acute stress induction on heart rate

The effect of acute stress induction on mean HR was tested using one-way rmANOVA (Session: RS0, RS1, adapted ScanSTRESS task and RS2). A significant effect of session was found ($F(1.37, 50.93) = 113.77$, $p < 0.001$). HR was significantly higher during the stress task than during RS0 (MD = 20.07 [95% CI = 16.30 – 23.83], $p < 0.001$), RS1 (MD = 19.44 [95% CI = 16.23 – 22.66], $p < 0.001$) and RS2 (MD = 15.03 [95% CI = 12.84–17.22], $p < 0.001$). HR was also higher during RS2 than during RS0 (MD = 5.03 [95% CI = 3.06 – 7.00], $p < 0.001$) and RS1 (MD = 4.41 [95% CI = 2.82 – 6.00], $p < 0.001$) (Figure S2).

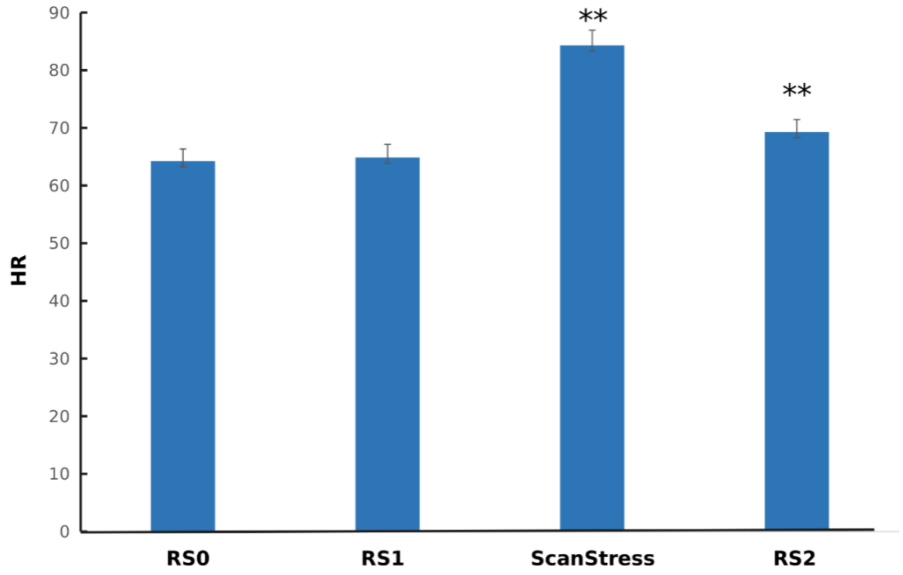


Figure S2: Effect of acute stress induction on heart rate (HR). HR was significantly higher during the stress task (adapted version of the ScanStress) compared to RS0 (MD= 20.07 [95% CI 16.30 – 23.83], p <0.001), RS1 (MD= 19.44 [95% CI 16.23 – 22.66], p < 0.001) and RS2 (MD= 15.03 [95% CI 12.84–17.22], p < 0.001). A significant increase in HR was also observed in RS2 compared to RS0 (MD= 5.03 [95% CI 3.06–7.00], p < 0.001) and RS1 (MD= 4.41 [95% CI 2.82–6.00], p < 0.001) condition. (RS0 = Baseline Session; RS1 = resting period between placebo and stress induction; ScanStress = During the ScanStress task; RS2 = after stress induction.).

3. The Carry-over Effect of Acute Stress Induction on resting-state functional connectivity

The carry-over effect of acute stress induction on resting-state functional connectivity (rsFC) was examined by a linear mixed model using the fitlme command in MATLAB. FC between network-pairs during the whole scan was used as the depended variable and session (RS0 and RS2), network-pair (DMN-SN, DMN-CEN, and SN-CEN) and session x network-pair were used as fixed effect terms, while subject was taken as random term. The full covariance matrix was chosen and the parameters were estimated by REML.

In baseline (RS0), rsFC between DMN-CEN was significantly stronger compared to both DMN-SN (t (210) = -2.47, p = 0.01) and SN-CEN (t (210) = -2.26, p= 0.02). After acute stress induction, rsFC between DMN-CEN (t (210) = -1.97, p = 0.05) was significantly reduced (Figure S3).

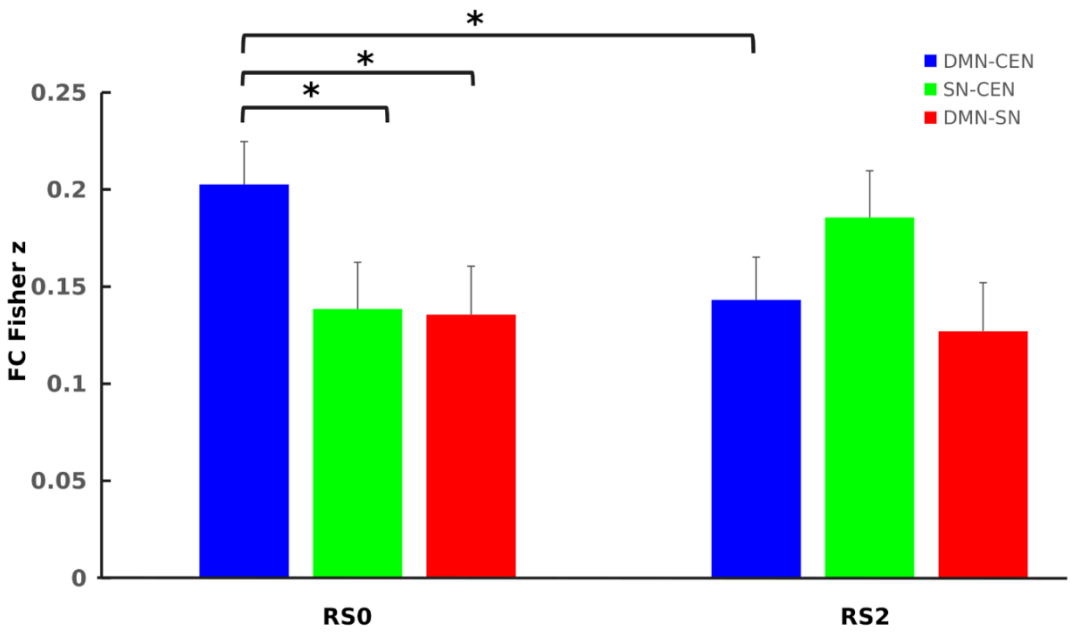
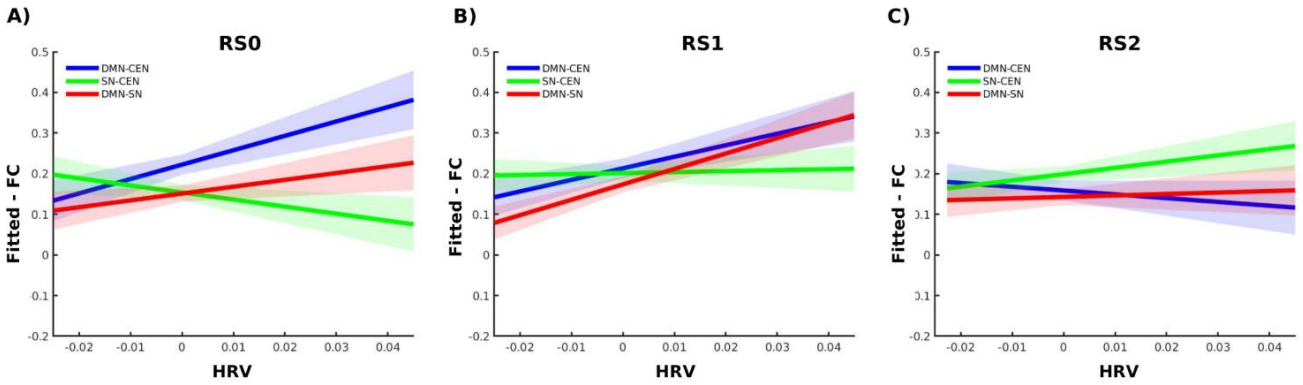


Figure S3: Resting-state functional connectivity (rsFC) between DMN-CEN, SN-CEN and DMN-SN at RS0 and RS**2**. In RS0, DMN-CEN showed a significantly stronger rsFC than DMN-SN and SN-CEN pairs. DMN-CEN rsFC was significantly reduced in RS2 as compared to RS0. (RS0 = Baseline Session; RS2 = After stress induction; SN = Salience Network; DMN = Default Node Network; CEN = Central Executive Network; * $p < 0.05$).

4. Dynamic temporal association between heart rate variability and dynamic functional connectivity between Network-pairs in RS1

This study included three resting state functional magnetic resonance imaging scans. The first one was acquired at baseline (RS0), the second one (RS1) was acquired after placebo intake and just before the ScanSTRESS task and the third one (RS') was acquired immediately after the ScanSTRESS task. Because placebo intake can induce changes in resting state brain activity (Tétreault et al., 2016; Wager et al., 2004), we restricted our main analyses to the comparison of RS0 and RS2. To explore the dynamic temporal association between HRV and dFC between network-pairs in RS1, additional analyses were built using a multilevel linear mixed model with a diagonal covariance matrix, where window-by-window FC between network-pairs was used as the dependent variable, while session (RS0, RS1 and RS2), network-pair (DMN-CEN, DMN-SN, and SN-CEN) and window-by-window HRV were added as regressors in parallel to the main analyses in the manuscript.



Comparable to the RS0 findings, also in RS1 window-by-window HRV was correlated to dFC between DMN-SN (b = 3.78, t(7641) = 4.45, $p <$ 0.001 (uncorrected) and DMN-CEN (b = 2.86, t(7641) = 3.37, $p < 0.001$ (uncorrected) FC with HRV in RS1 (Table S1). As depicted in Figure S4, the temporal association of SN-CEN dFC with HRV was significantly weaker than the association of DMN-SN dFC (b = -3.48, t(7641) = - 2.93, $p < 0.003$ (uncorrected) and DMN-CEN dFC with HRV (b = -2.68, t(7641) = -2.23, $p < 0.02$ (uncorrected) in RS1 as it was found in RS0 (Table S2).

After acute stress induction the association of HRV with DMN-CEN dFC (b= -3.78, t (7641) = -3.002, $p = 0.003$ (uncorrected)) was significantly weaker in comparison to RS1 as it was in comparison to RS0. In addition, the association between HRV and DMN-SN dFC (b = -3.42, t (7641) = -2.72, p= 0.006 (uncorrected)) was significantly decreased in RS1 in comparison to RS2 (Table S3).

There were no significant differences between RS0 and RS1 in terms of differential association of HRV and dFC between network-pairs (Table S3). These findings were also observed after controlling for the effect of age and the order of placebo intake.

Figure S4: Differential temporal association between heart rate variability (HRV) and functional connectivity (FC) between network-pairs across three sessions (RS0, RS1 and RS2). **A)** The multilevel linear mixed effect model showed a significant correlation between HRV and FC between DMN-CEN at baseline (RS0). The strength of association between HRV and dFC was significantly stronger for DMN-CEN than for SN-CEN in the baseline session (RS0). **B)** Pearson correlation between HRV and dFC DMN-SN and dFC DMN-CEN in RS1. The association between HRV and dFC was significantly stronger for DMN-CEN than for SN-CEN in RS1. **C)** The correlation between HRV and FC between DMN-CEN was significantly weaker in the second session (RS2) in comparison to RS0 and RS1. (RS0 = Baseline Session; RS1 = resting period between placebo and stress induction; RS2 = after stress induction; SN = Salience Network; DMN = Default Node Network; CEN = Central Executive Network; Shaded areas indicate standard error.).

Table S1: Association of heart rate variability with dynamic functional connectivity between network-pairs for each session (RS0, RS1 and RS2)

Session	FC	Estimate	SE	T	Unadjusted P-value	Adjusted P-value
RS0	DMN-SN	1.62	1.05	1.53	0.12	1.00
	DMN-CEN	3.50	1.05	3.33	**<0.001****	**<0.01****
	SN-CEN	-1.77	1.05	-1.68	0.092	0.82
RS1	DMN-SN	3.78	0.84	4.50	**<0.001*****	**< 0.01****
	DMN-CEN	2.86	0.85	3.37	**<0.001*****	**< 0.01****
	SN-CEN	0.26	0.84	0.31	0.759	1.00
RS2	DMN-SN	0.36	0.93	0.38	0.702	1.00
	DMN-CEN	-0.93	0.94	-0.99	0.319	1.00
	SN-CEN	1.54	0.93	1.65	0.09	0.81

Note: RS0 = Baseline Session; RS1= After placebo intake, before stress indcution; RS2 = After stress induction; SN = Salience Network; DMN = Default Node Network; CEN = Central Executive Network; Adjusted *p*-value= Bonferroni corrected *p*-values; Bold font represents significant results; * represents $p < 0.05$; ** represents $p < 0.01$.

Table S2: The within-session comparisons of correlation strengths between HRV and dFC between network-pairs

Session	Reference NP	Target NP	Estimate	SE	T	Unadjusted P-value	Adjusted P-value
RS0	DMN-SN	SN-CEN	-3.40	1.49	-2.28	**0.023***	0,18
	DMN-CEN	DMN-SN	-1.87	1.49	-1.25	0.210	1.00
	DMN-CEN	SN-CEN	-5.28	1.49	-3.54	**<0.001****	**<0.01****
RS1	DMN-SN	SN-CEN	-3.48	1.19	-2.93	**0.003****	**0.01****
	DMN-CEN	DMN-SN	1.19	1.20	1.00	0.318	1.00
	DMN-CEN	SN-CEN	-2.68	1.20	-2.23	**0.025***	0.22
RS2	DMN-SN	SN-CEN	1.18	1.32	0.89	0.371	1.00
	DMN-CEN	DMN-SN	1.28	1.32	0.97	0.330	1.00
	DMN-CEN	SN-CEN	2.47	1.32	1.87	0.062	0.54

Note: NP = Network-pair; RS0 = Baseline Session; RS1= After placebo intake, before stress indcution; RS2 = After stress induction; SN = Salience Network; DMN = Default Node Network; CEN = Central Executive Network; Adjusted P-value= Bonferroni corrected *p* values; Bold font represents significant results; * represents $p < 0.05$; ** represents $p < 0.01$.

Table S3: The between-session comparisons of correlation strengths between HRV and network-pairs dFC

NP	Reference Session	Target Session	Estimate	SE	T	Unadjusted P-value	Adjusted P-value
DMN-SN	RS0	RS1	2.17	1.35	1.61	107	0.96
	RS0	RS2	-1.26	1.41	-0.90	0.369	1.00
	RS1	RS2	-3.43	1.25	-2.73	**0.006****	**0.05***
DMN-CEN	RS0	RS1	-0.65	1.35	-0.48	0.631	1.00
	RS0	RS2	-4.44	1.41	-3.15	**<0.002****	**0.01****
	RS1	RS2	-3.79	1.26	-3.00	**<0.003****	**0.01****
SN-CEN	RS0	RS1	2.03	1.35	1.51	0.131	1.00
	RS0	RS2	3.31	1.41	2.35	**0.018***	0.16
	RS1	RS2	1.28	1.26	1.02	0.308	1.00

Note: NP = Network-pair; RS0 = Baseline Session; RS1= After placebo intake, before stress indcution; RS2 = After stress induction; SN = Salience Network; DMN = Default Node Network; CEN = Central Executive Network; Adjusted P-value= Bonferroni corrected *p* values; Bold font represents significant results; * represents $p < 0.05$; ** represents $p < 0.01$.

5. The distribution of the windowed time series for each network

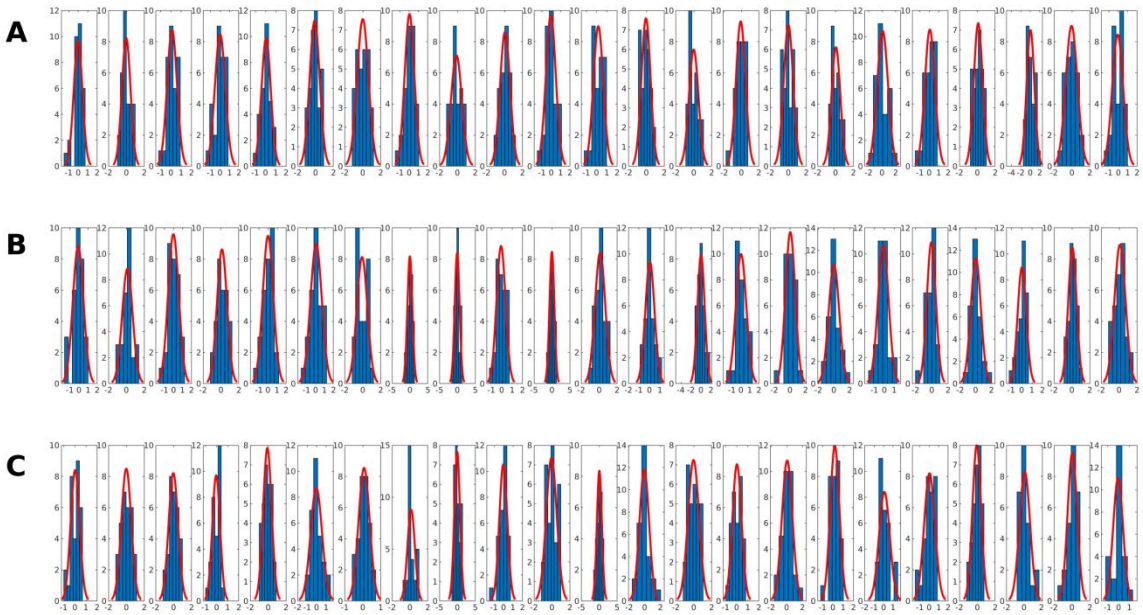


Figure S5: The distribution of the windowed time series for each network for a typical subject (P0010-ld98-010) of one resting-state session (RS0). Here each row represents each network (A). Default Mode Network (DMN), (b). Salience Network (SN), (c). Central Executive Network (CEN) and each column represents a window.

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