UNIVERSITY OF PADOVA

Department of General Psychology

Master's degree in Cognitive Neuroscience and Clinical Neuropsychology

Final dissertation

"Body and brain responses to acute psychosocial stress: physiological markers during the Trier Social Stress Test (TSST).**"**

"Risposte corporee e cerebrali allo stress psicosociale acuto: marcatori fisiologici durante il Trier Social Stress Test (TSST)"

Supervisor:

Professor Giovanni Mento

Co-supervisor:
Dr. Fiorella Del Popolo Cristaldi

Candidate: Navid Nadji

Student ID number: 2105923

Academic Year: 2025/2026

Table of Contents

Acknowledgements

I would first like to express my sincere gratitude to my supervisor, Professor Giovanni Mento, for his guidance and support throughout the development of this thesis. His expertise in cognitive neuroscience and his thoughtful feedback helped me shape the direction of this project and refine my scientific thinking. Beyond the scientific knowledge I gained during this process, I also learned from him the importance of academic rigor, critical thinking, and professionalism in research.

I would also like to give special thanks to my co-supervisor, Dr. Fiorella Del Popolo Cristaldi, for her invaluable mentorship during all phases of this work. She patiently taught me laboratory procedures and guided me step by step through participant data collection and analysis, and provided feedback on my thesis. Her dedication, availability, and attention to detail greatly improved my understanding of experimental research. I am grateful for the time she dedicated to reviewing my work and for the encouragement she provided during the different stages of this research. Beyond the technical aspects, I am especially thankful for her empathy and support during challenging moments throughout this journey.

I am deeply grateful to my family and friends for their constant encouragement, patience, and belief in me. Their emotional support gave me the strength and motivation to continue working toward my goals and reach this important milestone.

Finally, I would like to thank the University of Padova and the Department of General Psychology for providing the academic environment, facilities, and resources necessary to conduct this research. The opportunity to study and carry out research in such a stimulating scientific setting has been an important part of my academic growth.

This thesis represents not only my individual work, but also the support, guidance, and encouragement of many people who helped me along the way.

Abstract

Acute psychosocial stress engages coordinated changes across neural and autonomic systems. However, many studies examine isolated physiological signals or a single time period, limiting understanding of stress as a dynamic brain–body process unfolding across anticipation, exposure, and recovery phases. The present thesis investigated phase-resolved neural and autonomic responses to acute psychosocial stress using the Trier Social Stress Test (TSST) for induction of psychosocial stress within a multimodal framework integrating electroencephalography (EEG), electrocardiography (ECG), and electrodermal activity (EDA). High-density EEG and autonomic measures were recorded in healthy adults across the four phases of TSST. Oscillatory EEG power was quantified in canonical frequency bands (delta, theta, alpha, beta) across predefined scalp clusters (Frontal, Left Central, Right Central, Occipital). Autonomic indices included heart rate, heart rate variability, tonic skin conductance level, and phasic skin conductance responses. Phase and Cluster effects were examined using linear mixed-effects models, and brain–body associations were assessed via correlational analyses. Results revealed task-dependent modulation of both central and peripheral indices across systems. Sympathetic activation increased during the speech phase and then partially recovered thereafter. During the speech phase, beta power increased relative to baseline, whereas alpha power showed a distinct modulation pattern consistent with a decrease relative to baseline. In contrast, during the anticipation phase, alpha power showed an early decrease while beta activity began to increase, whereas during the recovery phase, alpha power tended to return toward baseline and beta power decreased, suggesting a gradual normalization of neural activity after the stressor. Critically, individual differences in neural oscillatory dynamics were systematically related to stress reactivity: lower alpha power was associated with stronger sympathetic activation and greater subjective anxiety, whereas greater beta increases were associated with heightened autonomic responses. By integrating central and peripheral physiological measures and considering individual differences, this thesis contributes to a multimodal understanding of the stress response.

Keywords: Acute psychosocial stress, TSST, Electroencephalography (EEG), Electrocardiography (ECG), Electrodermal activity (EDA), brain–body associations

Introduction

Given the repeatedly reported link between stress and disease, considerable research has focused on understanding how stress affects individuals and which stimuli can elicit a stress response (Biondi & Picardi, 1999). Stress can be defined as the mental and physical reaction to personal or environmental stimuli that are perceived as threatening to an individual (Folkman & Lazarus, 1985), While stress responses can facilitate effective behavior in the short term, prolonged or excessive activation of stress systems has been associated with a wide range of negative outcomes, including impairments in cognitive functioning, emotional regulation, and physical health (Lupien et al., 2009; McEwen & Morrison, 2013).

Acute stress is a common experience in everyday life and can be triggered by socially demanding situations such as examinations, public speaking, or job interviews, often leading to increased psychological tension and physiological activation (Sandi & Haller, 2015). Understanding the mechanisms underlying brain and body responses to stress is therefore crucial for advancing knowledge of stress regulation and its effects on human health (Lupien et al., 2009). Psychosocial stressors are particularly relevant in this context because they involve situations characterized by social evaluation, uncertainty, and performance pressure. These conditions can strongly activate stress-related physiological systems and produce measurable changes in both neural and autonomic activity, and cause issues for humans.

When a stressful stimulus occurs, both the brain and the body respond through coordinated neural and physiological processes. The stress response involves the coordinated activation of multiple biological systems, particularly the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis. The ANS contributes to rapid physiological adjustments through changes in heart rate, cardiac variability, and sweat gland activity, whereas the HPA axis supports a slower neuroendocrine response through the release of cortisol. Together, these systems prepare the organism to respond adaptively to environmental demands (Ulrich-Lai & Herman, 2009; McEwen & Morrison, 2013).

To study acute stress in laboratory settings, researchers use experimental tasks that induce psychosocial stress in a standardized and controlled way. One of the most widely used paradigms for this purpose is the Trier Social Stress Test (TSST). The TSST typically involves a speech and mental arithmetic task performed in front of an evaluative panel. This paradigm has been shown

to reliably elicit strong psychological and physiological stress responses, demonstrating high reliability and large effect sizes (Kirschbaum et al., 1993; Dickerson & Kemeny, 2004). Previous research suggests that cortical activity is related to autonomic regulation during the TSST (Thayer & Lane, 2000) and that acute stress can modulate oscillatory brain activity (Vanhollebeke et al., 2022).

For this reason, the present thesis aims to investigate how acute psychosocial stress modulates both central and peripheral physiological responses. Specifically, at the central level, the study examines changes in brain oscillatory activity, measured as power spectral density (PSD) using electroencephalography (EEG), one of the most widely used methods for assessing neural responses to stress. At the peripheral level, these neural measures are analyzed together with physiological indices, including electrocardiography (ECG), which reflects the electrical activity of the heart, and electrodermal activity (EDA), which reflects sympathetic nervous system (SNS) activation.

Although a growing number of studies have investigated neural or autonomic responses to stress separately, fewer studies have examined both systems simultaneously. However, stress is inherently a multisystem process involving interactions between central and peripheral physiological mechanisms (Ulrich-Lai & Herman, 2009). Investigating these systems together may therefore provide a more comprehensive understanding of how the brain and body coordinate responses to stressful situations. In addition, many studies have focused on overall stress responses without considering how these responses evolve across different phases of the stress experience. The acute stress response typically unfolds dynamically, including anticipation, exposure to the stressor, and recovery phases (Campbell & Ehlert, 2012). Furthermore, individual differences in stress reactivity may contribute to variability in neural and autonomic responses across participants, highlighting the importance of considering individual variability when investigating stress-related physiological processes. Based on previous literature on neural and autonomic responses to stress, findings on brain activity during the stress response remain heterogeneous, and the relationship between neural measures and physiological indices is still not fully understood. Therefore, further investigation is needed, which motivates the present thesis.

The first hypothesis concerns task-specific changes in neural activity. It is expected that EEG spectral power will vary across the different phases of the stress protocol, reflecting modulation of neural oscillatory activity during the baseline, anticipation, exposure, and recovery stages of the

stress response. The second hypothesis investigates individual differences in stress reactivity. Specifically, we examine whether variability in neural activity during acute psychosocial stress is associated with individual differences in sympathetic physiological responses.

Chapter One: The stress response

1.1 Definition of stress & psychosocial stress

Stress can be described as the perception that environmental or personal demands exceed an individual's capacity to cope, triggering an acute physiological response (Lazarus, 1966). In this context, stress does not refer to a single stimulus or physiological response, but to a dynamic process determined by the interaction between external demands and individuals' appraisals of those demands. This process gives rise to coordinated psychological and physiological adjustments aimed at supporting adaptation and maintaining functional stability (McEwen, 2007). From this perspective, a stressor is any internal or external factor perceived as challenging an individual's capacity to maintain balance. This perception triggers a stress response, defined as the coordinated activation of biological and psychological processes intended to maintain internal stability (Kirschbaum et al., 1993).

Psychosocial stress is a subtype of stress that occurs during social interactions. It commonly occurs from stressful stimuli associated with novelty, unpredictability, lack of control, or social evaluation (Vanhollebeke et al., 2022). Unlike physical stressors, psychosocial stressors rely primarily on cognitive appraisal and social context rather than direct physical harm. Cognitive appraisal refers to the process by which individuals evaluate and interpret a situation in relation to their well-being. According to the transactional model of stress (Folkman & Lazarus, 1985), stress responses depend on how a person appraises a potential stressor. During primary appraisal, individuals assess whether a situation is irrelevant, benign, threatening, or challenging. During secondary appraisal, they evaluate their available coping resources and their ability to manage the demands of the situation. These appraisal processes influence the intensity of the stress response and shape both psychological reactions and physiological activation (Folkman & Lazarus, 1985). These stressors are particularly potent because they tap into core psychological needs, triggering both emotional arousal and physiological responses even in the absence of physical danger. Stressors strongly engage brain processes involved in evaluation and anticipation, as well as bodily systems that support adaptive responses. For this reason, psychosocial stress provides an ecologically valid

framework for studying brain–body interactions during stress (Folkman & Lazarus, 1985; McEwen, 2007).

In addition to differences in stressor type, stress can also be classified based on its temporal characteristics, most commonly distinguishing between acute and chronic stress**.** Acute stress refers to a short-term physiological and psychological response elicited by a time-limited stressor, characterized by quick activation of autonomic and neuroendocrine systems that typically return to baseline once the stressor is removed (McEwen, 2007).

Chronic stress refers to prolonged or repeated exposure to stressors that persist over time, particularly in situations where individuals perceive limited control or insufficient coping resources (McEwen, 2007). Prolonged or repeated activation of stress responses can have cumulative effects that go beyond their immediate adaptive function. This cumulative physiological strain is captured by the concept of allostatic load, introduced by McEwen and Stellar (1993). Allostatic load refers to the long-term physiological strain on the body and brain resulting from repeated or chronic activation of neural, autonomic, and neuroendocrine stress systems. Over time, increased allostatic load reflects dysregulation across multiple biological systems and contributes to raised vulnerability to stress-related physical and mental health outcomes.

The relationship between stress level and performance is often described by the Yerkes–Dodson law, which posits an inverted U-shaped relationship between arousal and performance, as shown in Figure 1 (Yerkes & Dodson, 1908).

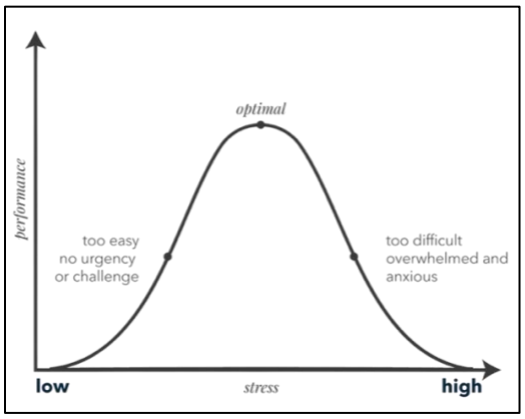


Figure 1. *Inverted U-shaped relationship between stress and performance*

According to this model, performance tends to improve as stress or arousal increases from very low levels, because moderate activation of the nervous system enhances alertness, attention, and task engagement. When stress levels are too low, individuals may experience reduced motivation and insufficient cognitive activation, which can lead to poorer task performance. As arousal rises to moderate levels, physiological activation supports cognitive processes such as vigilance, information processing, and goal-directed behavior, allowing performance to reach an optimal level. However, when stress exceeds this optimal point, performance typically begins to decline. Consequently, while moderate stress may facilitate adaptive functioning, excessive stress can impair both cognitive and behavioral performance (Lupien et al., 2009).

1.1.1 Stress reactivity

Stress reactivity refers to how strongly an individual responds when exposed to a stressor, across psychological experience and physiological systems. In other words, two people can face the same stressful situation, but show different "response amplitudes" in measures. Importantly, stress reactivity is not identical to self-reported stress: people can report feeling highly stressed while showing relatively modest physiological activation. This partial dissociation suggests that subjective and physiological responses reflect not identical aspects of the stress response (Campbell & Ehlert, 2012). In laboratory research, stress reactivity is typically described as the change from a baseline state to a stress state, capturing the magnitude of stress-related activation,

while recovery reflects the extent and speed with which these responses return toward baseline once the stressor ends (Dickerson & Kemeny, 2004; McEwen, 2007).

An additional important characteristic of stress reactivity concerns not only the magnitude of the response but also its duration. An adaptive stress response is typically characterized by a temporary increase in physiological activation that returns to baseline once the stressor has ended. However, when stress responses remain elevated for longer periods, this prolonged activation can place greater physiological demands on the body and contribute to allostatic load over time. In this sense, both exaggerated responses and delayed recovery may represent maladaptive patterns of stress reactivity that increase vulnerability to negative health outcomes (Kiecolt-Glaser et al., 2020). Stress reactivity involves coordinated changes across multiple physiological systems rather than occurring within a single biological domain. In response to a stressor, the body activates cardiovascular, endocrine, autonomic, and immune processes that prepare the organism to respond to environmental demands. Therefore, stress reactivity should be understood as a multidimensional process that reflects the integrated functioning of several physiological systems (Kiecolt-Glaser et al., 2020).

Individual differences in stress reactivity have been consistently linked to stable psychological traits. Higher stress reactivity has been associated with elevated trait anxiety and neuroticism, as well as greater negative affectivity (Bolger & Schilling, 1991; Schlotz et al., 2011). Individuals with high trait anxiety tend to appraise situations as more threatening and often show amplified physiological and subjective responses during laboratory stress tasks. Similarly, greater neuroticism has been related to stronger emotional and, in some cases, autonomic responses to stress. Conversely, lower stress reactivity has been associated with higher levels of resilience, perceived control, and effective emotion regulation abilities (Seery, 2011; Tugade & Fredrickson, 2004). Individuals who report greater coping resources or adaptive appraisal styles often display attenuated physiological activation or faster recovery following stress exposure. Importantly, the biopsychosocial model of challenge and threat (Blascovich et al., 1999) emphasizes that stress responses depend not only on the external stressor but also on how the situation is appraised. When individuals perceive that their coping resources are sufficient, a challenge state emerges, typically associated with more adaptive psychological and physiological patterns. In contrast, when perceived resources are insufficient, a threat state develops, often linked to heightened anxiety and

less efficient regulatory responses. These distinctions highlight the central role of appraisal in shaping both psychological experience and physiological activation.

Evidence suggests that intermediate stress responses may be the most adaptive, whereas both exaggerated and blunted physiological reactions may increase vulnerability to future health problems.

1.1.2 Stress recovery

Stress recovery refers to the process through which psychological and physiological responses decline after the stressor has ended and gradually return toward baseline levels. While stress reactivity reflects the magnitude of activation during a stressful event, recovery captures how efficiently the organism deactivates once the demand is no longer present. For this reason, recovery is not simply the opposite of reactivity, but a distinct phase of the stress response that provides important information about regulatory capacity (Dickerson & Kemeny, 2004; McEwen, 2007). In laboratory paradigms, recovery is typically assessed by examining whether measures such as cortisol, heart rate, heart rate variability, and subjective stress return toward pre-stress levels during the post-task period.

Efficient recovery is generally considered an adaptive feature of the stress response. A temporary rise in physiological activation can be beneficial. However, once the stressor has passed, these systems should downregulate. When activation remains elevated for too long, prolonged exposure may increase allostatic load, that is, the cumulative biological burden associated with repeated or sustained stress responses over time (McEwen, 1998). Delayed recovery has therefore been linked to a greater risk of negative mental and physical health outcomes, including anxiety, depression, cardiovascular dysregulation, and stress-related allostatic load across bodily systems (Burke et al., 2005; Fiksdal et al., 2019; Turner et al., 2020).

As with reactivity, stress recovery is a multidimensional process. Different physiological systems recover at different speeds and may not return to baseline in a fully synchronized way. For example, autonomic indices such as heart rate may normalize relatively quickly after stress cessation, whereas cortisol responses typically peak later and recover more gradually because of

the slower dynamics of HPA-axis activation. Subjective feelings of stress may follow yet another trajectory, sometimes decreasing before endocrine markers have fully recovered. This temporal dissociation suggests that recovery should not be understood as a single uniform process, but rather as a set of partially independent trajectories across subjective, autonomic, and endocrine domains (Campbell & Ehlert, 2012; Fiksdal et al., 2019).

Recent work has further suggested that recovery may be especially informative for understanding resilience. A resilient physiological profile is often characterized not only by low or moderate reactivity, but also by relatively rapid recovery following stress exposure. In this view, recovery reflects the flexibility of stress-regulatory systems and their capacity to restore equilibrium after challenge. Supporting this idea, a physiological resilience index (Liang et al., 2026) was developed based on TSST responses combining cortisol, heart rate, and blood pressure. Their results showed that lower stress reactivity and faster recovery were associated with lower psychological distress. These findings suggest that stress recovery is an important indicator of adaptive stress regulation and should be considered separately from stress reactivity.

1.2 Psychological Components of the Stress Response

At **t**he psychological and behavioral level, stress is characterized by increased arousal, heightened vigilance, and changes in emotional state, particularly anxiety and tension. However, stress is not simply a rise in activation; it reflects an ongoing process of evaluation. According to transactional appraisal theory (Folkman & Lazarus, 1985), individuals continuously assess whether environmental demands exceed their available coping resources. This evaluative process shapes emotional reactions, attentional focus, and behavioral strategies aimed at managing the situation.

In the context of acute psychosocial stress, such as socially evaluative situations, individuals often experience increased state anxiety, stronger self-focused attention, and enhanced monitoring of social cues. When performance is observed and judged by others, emotional responses become more intense, and attention becomes more sensitive to potential threat (Dickerson & Kemeny, 2004). Under these conditions, cognitive processing tends to prioritize threat-related information, leading to a narrowing of attentional focus and faster, stimulus-driven decision-making, with reduced deliberation and increased reliance on automatic responses (Eysenck et al., 2007). While

this shift may support rapid coping in the short term, it can reduce cognitive flexibility and broader information processing. Acute stress can also temporarily impair executive functions such as working memory, inhibitory control, and cognitive flexibility, especially when perceived demands exceed coping capacity (Arnsten, 2009; Shields et al., 2016). These changes are associated with reduced efficiency in prefrontal regulatory systems, increasing reliance on more automatic or habitual responses. Although such responses may be adaptive when quick reactions are required, prolonged exposure to stress may compromise careful reasoning and long-term planning.

Beyond changes in attention and executive function, stress also influences motivational and emotional processing. Individuals under stress show increased sensitivity to emotionally salient or threatening stimuli (Pessoa, 2009). This heightened sensitivity may contribute to sustained vigilance and stronger emotional reactivity during socially demanding tasks. At the same time, stress can shift behavior from goal-directed control toward more habitual or reward-driven strategies (Schwabe & Wolf, 2013). While this shift may facilitate performance under pressure, it may reduce behavioral flexibility when adaptation is necessary.

Finally, psychological responses to stress change dynamically over time. Subjective anxiety often increases during the anticipation phase, as individuals evaluate the upcoming stressor and its potential consequences. Importantly, the magnitude of this anticipatory anxiety varies across individuals and is associated with stable traits, such as trait anxiety and differences in emotion regulation capacity (Schüle et al., 2007). Following stress exposure, subjective anxiety may decrease, although recovery patterns differ across individuals. Together**,** these findings highlight that the psychological stress response reflects a dynamic process involving anticipatory appraisal, emotional engagement, and subsequent recovery (Dickerson & Kemeny, 2004; Mcewen, 2007).

Overall, the psychological stress response reflects coordinated changes in cognition, emotion, and behavior driven by the appraisal of environmental demands and coping resources. Rather than being static, these responses vary across individuals and across phases of the stress experience, highlighting the central role of appraisal in shaping how stress is perceived and regulated.

1.3 Central & peripheral mechanisms of stress

In this section, the physiological mechanisms underlying the stress response are discussed from both peripheral and central perspectives. Specifically, the following subsections review the role of the autonomic nervous system (ANS), as part of the peripheral nervous system, and the activity of the central nervous system, focusing on brain dynamics associated with stress processing.

1.3.1 Peripheral & autonomic responses to stress

Selye (1965) described the stress syndrome as a coordinated set of physiological responses aimed at protecting the organism when it is exposed to demanding or threatening conditions. According to this view, the stress response involves the activation of two main physiological systems. The sympathetic–adreno–medullary (SAM) axis provides a rapid response, often referred to as the "fight-or-flight" reaction, which occurs within seconds and is characterized by the release of adrenaline that increases heart rate and mobilizes energy resources. In parallel, the hypothalamic-pituitary-adrenal (HPA) axis generates a slower, more sustained response through cortisol release, supporting adaptation when demands persist over time (Selye, Hans, 1965).

A key system underlying these stress responses is the ANS, which regulates physiological arousal through the interaction of two branches: the sympathetic nervous system, which increases arousal, and the parasympathetic nervous system, which reduces it. During physical or psychological stress, sympathetic activity becomes dominant, leading to increased arousal and a faster heart rate that helps the body respond to the challenge. Whereas, during periods of safety and stability, parasympathetic activity predominates, maintaining lower levels of arousal and a slower heart rate. The ability to transition efficiently between high and low arousal states depends on the flexibility of the autonomic nervous system and the organism's broader regulatory capacity across multiple physiological systems (McEwen, 1998). The HPA axis is activated when stress-related signals reach the hypothalamus, leading to the release of corticotropin-releasing hormone (CRH). The release of CRH into the pituitary portal system induces the pituitary to release adrenocorticotropic hormone (ACTH), which in turn induces the release of glucocorticoids (GCs) from the adrenal cortex. GCs exert negative feedback on the hypothalamus and pituitary gland, which serve to terminate the stress response when no longer required, thereby preventing excessive responses.

GCs are involved in many aspects of the stress response; they facilitate adaptation of the body to changing conditions by regulating energy stores, inhibiting nonessential physiological activity, and promoting behavioral responses to stimuli perceived as stressful (Herman et al., 2016; Johnson et al., 1992).

In addition to rapid autonomic responses, the endocrine component of the stress system plays an important role in sustaining physiological adaptation to stress. In particular, the hormone cortisol represents the main hormonal output of the HPA axis. Cortisol plays a key role in mobilizing energy resources, modulating immune function, and influencing cognitive processes through its widespread effects on the brain, particularly in regions involved in emotion regulation and decision-making (Herman et al., 2016). Cortisol secretion also follows a circadian rhythm, with levels typically peaking shortly after awakening and gradually declining throughout the day. This daily pattern is an important factor to consider when studying cortisol responses to stress (Adam & Kumari, 2009). Unlike the rapid autonomic responses mediated by the SAM axis, cortisol responses develop more gradually and typically peak several minutes after the onset of a stressor. In parallel, the sympathetic nervous system is rapidly engaged, leading to the release of catecholamines (adrenaline and noradrenaline) from the adrenal medulla and sympathetic nerve terminals. This autonomic activation produces immediate physiological changes, including increased heart rate, elevated blood pressure, enhanced sweat gland activity, and redistribution of blood flow toward muscles and vital organs. These peripheral responses are commonly indexed through measures such as heart rate, blood pressure, and electrodermal activity and reflect the organism's readiness for action (Ulrich-Lai & Herman, 2009).

In addition to autonomic effects, stress triggers neuroendocrine changes that support energy mobilization and sustained adaptation. Glucocorticoid release increases the availability of glucose by influencing metabolic processes in the liver, muscles, and adipose tissue, ensuring that sufficient energetic resources are available to meet increased physiological and cognitive demands during stress exposure (McEwen, 2007). While short-term increases in cortisol are adaptive, prolonged activation of the HPA axis may contribute to negative health outcomes, including metabolic, cardiovascular, and psychological disturbances (McEwen, 2007). These endocrine responses typically unfold on a slightly slower timescale than autonomic changes but contribute to maintaining the stress response when demands persist. Cortisol also regulates the stress response

through a negative feedback mechanism that reduces further activation of the HPA axis once sufficient hormone levels are reached, helping the organism return to physiological balance (De Kloet et al., 2005a).

Stress-related physiological activation also interacts with immune function. Acute stress can transiently modulate immune activity, often enhancing certain aspects of immune surveillance, whereas prolonged or repeated activation may lead to immune dysregulation (McEWEN, 1998). Importantly, the physiological stress response is inherently dynamic: regulatory mechanisms, including hormonal negative feedback and autonomic balance, normally promote recovery and a return to baseline once the stressor has been resolved (Ulrich-Lai & Herman, 2009).

1.3.2 Central nervous system responses to stress

The activation of the stress response is accompanied by pronounced changes within the central nervous system (CNS). Neural activity associated with stress can be investigated using several neuroimaging and neurophysiological techniques, including functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and positron emission tomography (PET). The fMRI measures changes in blood oxygenation associated with neural activity, known as the blood-oxygen-level-dependent (BOLD) signal, allowing researchers to identify brain regions with high spatial resolution (Logothetis, 2008). Positron emission tomography (PET) measures metabolic activity or neurotransmitter binding in the brain through the use of radioactive tracers (Phelps, 2004). Electroencephalography (EEG), in contrast, records the electrical activity generated by neuronal populations with high temporal resolution. Together, these methods provide complementary information about brain function, allowing researchers to examine both the spatial localization and temporal dynamics of neural activity during stress.

The CNS, defined as the part of the nervous system that comprises the brain and spinal cord, serves as the primary integrative and control center of the body. It plays a vital role in detecting stressors, evaluating their significance, and coordinating appropriate physiological and behavioral responses. Stress-related neural activity primarily involves a network of cortical and subcortical regions that support threat detection, emotional regulation, cognitive control, and interoceptive processing (McEwen, 2007; Ulrich-Lai & Herman, 2009).

Within subcortical structures, the amygdala, an almond-shaped group of nuclei located in the medial temporal lobe, plays a central role in detecting threats and generating emotional responses to stress (LeDoux, 2000). Increased amygdala activity has been consistently associated with elevated emotional arousal and with the facilitation of autonomic and neuroendocrine stress responses through its connections with hypothalamic and brainstem nuclei (Herman et al., 2016; LeDoux, 2000). In contrast, the hippocampus contributes to contextual processing and memory formation during stress and plays an important role in regulating the stress response by inhibiting the HPA axis via inhibitory feedback mechanisms. Stress-related elevations in glucocorticoids can transiently modulate hippocampal function, influencing learning and memory processes (McEwen, 2007).

At the cortical level, the prefrontal cortex (PFC), the anterior portion of the frontal lobes located at the front of the brain, plays a key role in higher-order cognitive functions, including decision-making, attention, and emotion regulation. Acute stress has been shown to alter PFC activity, often reducing top-down regulatory control over subcortical regions, including the amygdala (Arnsten, 2009). This shift may promote rapid, habitual, or emotionally driven responses at the expense of flexible cognitive control. While such changes can be adaptive in the short term, facilitating rapid responses to threats, prolonged or repeated stress exposure may impair prefrontal functioning and cognitive performance (Arnsten, 2009). Neural oscillations represent temporally coordinated patterns of activity that support large-scale communication across brain networks. Changes in oscillatory power during stress are therefore not merely epiphenomenal, but may reflect shifts in attention, inhibitory control, motivational salience, and regulatory engagement (Arnsten, 2009; Jensen & Mazaheri, 2010). Investigating oscillatory dynamics provides a window into how stress reorganizes cortical processing in real time.

1.3.3 Brain–Body Relationships in Stress

Brain–body relationships refer to the dynamic and bidirectional coordination between central neural activity and peripheral physiological systems during adaptive responses. In the context of stress, this coupling reflects the continuous interaction between cortical and subcortical brain networks involved in evaluation and regulation, and autonomic and endocrine systems responsible

for mobilizing bodily resources. Rather than functioning as independent components, the brain and body operate as an integrated system in which neural processes influence autonomic output, while peripheral signals simultaneously shape neural activity through ascending interoceptive pathways (Craig, 2002; Ulrich-Lai & Herman, 2009). Acute psychosocial stress provides a particularly informative model for examining brain–body coupling because it simultaneously engages cognitive appraisal, emotional processing, and autonomic activation. Neural changes in oscillatory activity, especially within frontal and midline regions, have been shown to covary with cardiovascular and electrodermal responses, suggesting coordinated central–peripheral dynamics rather than isolated activation within a single system (Hermans et al., 2014; Vanhollebeke et al., 2022).

1.4 Theoretical Models of Stress

This section reviews theoretical frameworks that describe how stress responses emerge and are regulated across brain and body systems. First, the General Adaptation Syndrome (GAS) model is introduced as a classic physiological framework that explains the temporal stages of the stress response. Second, more recent interoceptive and predictive processing accounts are discussed, which provide a mechanistic perspective on how the brain monitors internal bodily signals and regulates autonomic responses during stress. Together, these perspectives help explain the dynamic interaction between central and peripheral physiological processes involved in stress regulation.

1.4.1 General Adaptation Syndrome (GAS)

One of the most fundamental and earliest conceptual models of stress is the General Adaptation Syndrome (GAS), proposed by Hans Selye (1965). GAS conceptualizes stress as a non-specific biological response of the organism to any demand placed upon it and describes this response as unfolding across three sequential stages, shown in Figure 2. The first stage is the alarm reaction, which occurs immediately after exposure to a stressor. During this phase, the body activates autonomic and endocrine responses, most notably the sympathetic nervous system and the HPA axis, to support heightened alertness, energy mobilization, and behavioral readiness. In the alarm stage, the physiological reaction closely resembles the classic “fight-or-flight” response originally

described by Cannon, in which the sympathetic nervous system rapidly prepares the organism to respond to potential threats (Cannon, 1932). This response involves the release of catecholamines such as adrenaline and noradrenaline, which increase heart rate, blood pressure, and energy availability to support immediate action. As stress exposure continues, glucocorticoids such as cortisol become more prominent in sustaining metabolic resources and supporting adaptation during the resistance stage.

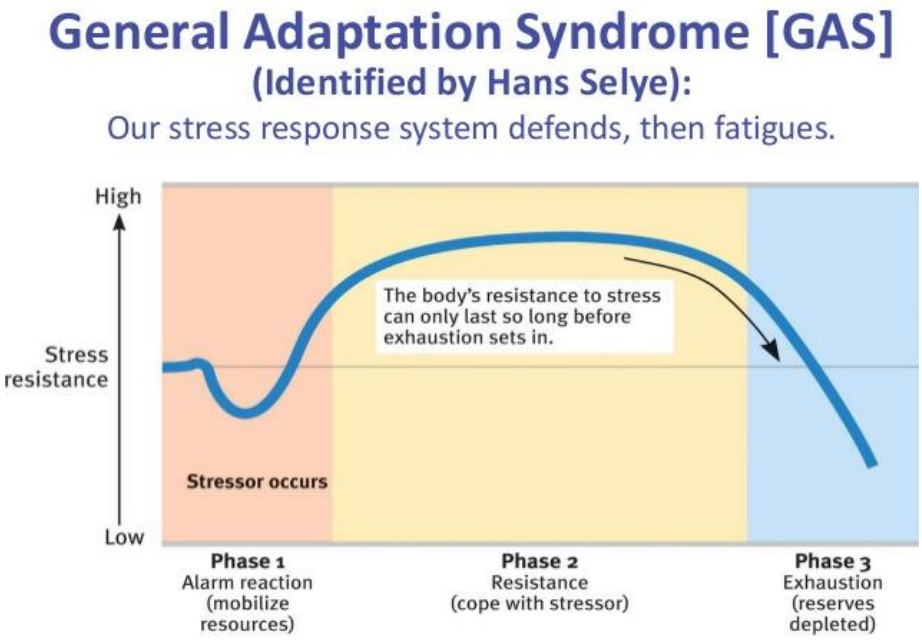


Figure 2. *Three Phases of General Adaptation Syndrome by Hans Selye*

The second stage is the resistance (or adaptation) phase. During this stage, the organism attempts to cope with the continued presence of the stressor by sustaining physiological activation while maintaining relative functional stability (Selye, Hans, 1965). Although the acute alarm response is no longer at its peak, stress-related physiological activity remains elevated as the body adapts to the continued demands. Hormones such as cortisol help mobilize metabolic resources, regulate immune activity, and maintain energy availability necessary for coping with the stressor. As a result, physiological activity remains elevated compared with baseline levels, but the body attempts to stabilize its functioning and prevent excessive depletion of resources.

The final stage is exhaustion, which reflects a breakdown of adaptive capacity following prolonged or excessive stress exposure. At this stage, resistance is diminished, physiological resources are depleted, and vulnerability to dysfunction, illness, or collapse increases (Selye, Hans, 1965). According to the GAS, very different stressors can evoke a similar pattern of physiological responses, reflecting the existence of a general, non-specific biological response to stress. In this sense, GAS provides a useful temporal framework for describing how stress responses unfold over time across the stages of alarm, resistance, and exhaustion (Selye, Hans, 1965). However, while the GAS primarily characterizes the temporal structure of stress responses, interoceptive theories offer a complementary mechanistic framework explaining how the brain monitors, predicts, and regulates internal bodily states across these phases.

1.4.2 Interoceptive and predictive framework

Interoception refers to the process by which the nervous system senses, interprets, and integrates signals originating from within the body, including physiological signals such as heart rate, breathing, and visceral activity (Craig, 2002; Khalsa et al., 2018). These internal bodily signals are continuously monitored by the brain and contribute to the perception of physiological states and emotional experiences.

Although the physiological mechanisms underlying stress responses are well established, growing evidence indicates that interoception plays a central role in shaping how stress is experienced, regulated, and recovered from. Predictive interoceptive theories (Barrett & Simmons, 2015; Seth, 2013) propose that the brain is not a passive receiver of bodily signals, but actively generates predictions about internal states and compares them with incoming interoceptive input. Discrepancies between predicted and actual bodily states, referred to as interoceptive prediction errors, signal the need for regulatory adjustment.

These accounts provide a mechanistic account of anticipatory stress, whereby the anticipation of a demanding or threatening event can shift interoceptive predictions even before the stressor occurs, leading to preparatory changes in neural and autonomic activity. As a result, physiological responses may emerge during anticipation rather than only during the stressor itself, reflecting the brain's attempt to minimize expected future prediction errors. Effective recovery occurs when

interoceptive prediction errors are reduced, and internal bodily states are successfully brought back into alignment with expectations. In contrast, delayed or incomplete recovery may reflect persistent mismatches between predicted and actual bodily signals, indicating ongoing dysregulation of interoceptive control mechanisms (Seth, 2013). Predictive models of interoception propose that individuals differ in how they anticipate and regulate internal bodily signals, due to variability in interoceptive priors and the weighting of prediction errors. As a result, exposure to the same stressor can produce variability in autonomic responses, subjective stress experiences, and recovery trajectories.

In this way, such models provide a mechanistic account of individual differences in stress reactivity and resilience (Khalsa et al., 2018; Seth, 2013). Although stress paradigms reliably induce group-level effects, individuals differ substantially in the magnitude and coordination of their neural and autonomic responses. Such variability reflects differences in appraisal processes, regulatory capacity, and interoceptive sensitivity, and may manifest as exaggerated, blunted, or prolonged physiological responses (Campbell & Ehlert, 2012; McEwen, 2007). Examining these individual differences is essential for understanding vulnerability and resilience in stress regulation.

1.5 Effects of stress on health

Stress is an inherent aspect of human life that significantly affects both physiological and neurological functioning. It can impair executive function and, in severe cases, may contribute to increased mortality. Individuals exposed to stressful family environments or workplaces are at increased risk for a range of disorders (Yaribeygi et al., 2017).

Stress research has become increasingly central in contemporary health science because of its substantial impact on global health and well-being. Recent work emphasizes that stress represents a major public health challenge, placing a growing burden on individuals and societies through its pervasive psychological and physiological effects (McEwen & Akil, 2020). In modern contexts marked by rapid technological change, economic instability, social isolation, and large-scale disruptions such as the COVID-19 pandemic, individuals are exposed to both acute and chronic psychosocial stressors with increasing frequency (Pfefferbaum & North, 2020). This sustained

exposure is associated with a broad range of adverse outcomes, including increased risk of cardiovascular disease, diabetes, chronic inflammation, anxiety, and burnout, underscoring the need for rigorous investigation of how stress develops, persists, and can be effectively mitigated (Selye, Hans et al., 2024). During stress, humans experience a decline in executive functioning, with research showing that reaction times increase and cognitive load is significantly higher during stress induction (Angioletti et al., 2025).

Physiological responses to acute stress may also provide important information about long-term health risk. Research suggests that individual differences in stress reactivity can predict vulnerability to future physical and psychological disorders. Individuals who consistently show stronger cardiovascular, endocrine, or inflammatory responses during acute stress tasks appear to have a greater likelihood of developing conditions such as hypertension and metabolic disturbances later in life (Steptoe et al., 2007; Turner et al., 2020). Importantly, maladaptive stress responses are not limited to exaggerated activation. Some individuals exhibit blunted physiological responses, characterized by unusually low cardiovascular or hormonal reactions to stressors. Although such responses might initially appear protective, research suggests that they may reflect reduced sensitivity of stress-response systems and have been linked to negative outcomes such as reduced behavioral engagement, impaired emotional regulation, and mental health vulnerabilities (Miller et al., 2007; Turner et al., 2020).

Another important factor influencing the health effects of stress involves psychological processes such as depression, worry, and rumination. These factors can intensify stress responses by prolonging physiological activation before and after a stressful event. In addition, experiences of adversity early in life can shape the development of stress-response systems, making individuals more vulnerable to stronger inflammatory, hormonal, and autonomic reactions to stress later in life (Kiecolt-Glaser et al., 2020).

Recognizing neuroplastic changes is critical for both prevention and treatment. While chronic stress fosters vulnerability to mood and anxiety disorders, targeted interventions such as mindfulness, stress inoculation training, and cognitive-behavioral therapy can enhance prefrontal control and support adaptive plasticity (McEwen & Morrison, 2013). In addition to these functional changes, chronic stress can also affect the structure of the brain. Prolonged exposure to

stress hormones such as cortisol has been associated with reductions in hippocampal volume, alterations in the prefrontal cortex, and increased amygdala reactivity (McEwen & Morrison, 2013). These regions are essential for memory, emotional regulation, and executive control. Over time, such structural changes may weaken the brain's ability to regulate stress effectively, increasing vulnerability to both psychological and physical health problems.

In addition, when the body remains in a prolonged state of physiological activation, increased sympathetic activity and elevated cortisol levels can disturb normal sleep patterns and circadian rhythms. As a result, individuals under high stress often experience difficulties falling asleep, frequent awakenings, or reduced sleep quality. Poor sleep can then further increase stress vulnerability by impairing emotional regulation, attention, and cognitive performance. This creates a bidirectional relationship in which stress disrupts sleep, and insufficient sleep makes individuals more sensitive to stress (Medic et al., 2017; Meerlo et al., 2008).

Stress responses are closely linked to immune regulation. During stress, activation of the HPA axis leads to the release of cortisol, a hormone that plays an important role in regulating inflammatory processes and maintaining immune balance. Under normal conditions, cortisol helps control excessive immune activity. However, when cortisol responses become dysregulated or prolonged, this regulatory mechanism may be disrupted, potentially leading to altered inflammatory activity and increased vulnerability to disease (Dhabhar, 2014). Additionally, stress responses may also influence musculoskeletal health. Evidence suggests that altered cortisol reactivity can affect physiological processes related to pain perception and bone metabolism. In particular, blunted cortisol responses to stress have been associated with a higher prevalence of musculoskeletal pain and lower bone mineral density. Insufficient hormonal responses during stress may therefore disrupt normal physiological regulation and contribute to increased vulnerability to pain disorders and bone-related conditions over time (Rohleder, 2019).

Chronic stress can also affect gastrointestinal health through the brain–gut axis, a bidirectional communication system linking the central nervous system and the digestive system. Through neural, hormonal, and immune pathways, stress can influence gut functioning by altering gastrointestinal motility, increasing intestinal permeability, and affecting the composition of the gut microbiome. These physiological changes may contribute to digestive symptoms such as

abdominal pain, discomfort, and altered bowel habits. As a result, chronic stress has been associated with a higher prevalence of gastrointestinal disorders, including conditions such as irritable bowel syndrome and functional dyspepsia (Mayer, 2011; Moloney et al., 2014).

Chronic stress has also been linked to metabolic disturbances. Prolonged activation of the HPA axis increases cortisol levels, which can affect glucose metabolism, promote insulin resistance, and contribute to the accumulation of visceral fat. In addition to these physiological mechanisms, stress may also influence metabolic health through behavioral factors such as emotional eating and reduced physical activity. Over time, these processes may increase the risk of obesity, metabolic syndrome, and type 2 diabetes (Chrousos, 2009; Kyrou & Tsigos, 2009).

Stress is therefore associated with a wide range of health issues affecting multiple physiological and psychological systems, including cognitive functioning, cardiovascular regulation, immune processes, sleep, and gastrointestinal health. Given the broad impact of stress on these systems, studying stress responses across both neural and autonomic levels is essential for developing a more comprehensive understanding of how the brain and body react to stressful situations.

Chapter Two: Experimental Paradigms & Psychophysiological Measures

2.1 Experimental stress induction paradigms

Experimental stress paradigms are designed to safely and systematically elicit acute stress within controlled laboratory environments. These experimental models enable researchers to investigate how psychological challenges translate into physiological and neuroendocrine responses, helping to clarify the causal pathways that underlie stress-related processes (Kudielka et al., 2007). In such paradigms, stress responses are typically triggered by factors such as uncontrollability, social evaluation, and cognitive load, all of which activate the HPA axis and the sympathetic nervous system (Dickerson & Kemeny, 2004).

Several well-established experimental protocols have been developed to induce stress in a controlled laboratory environment. One commonly used paradigm is the Maastricht Acute Stress Test (MAST), which combines exposure to cold water with cognitively demanding mental arithmetic tasks performed under time pressure and observation (Smeets et al., 2012). Participants are typically asked to repeatedly immerse their hand in ice-cold water while simultaneously performing difficult subtraction tasks, often while receiving negative feedback from the experimenter. This combination of physical discomfort, cognitive load, and social evaluation reliably induces acute stress responses. Another widely used protocol is the Socially Evaluated Cold Pressor Test (SECPT). In this task, participants are required to immerse their hand in ice water for a fixed period while being observed or filmed by an experimenter. The presence of social evaluation increases the perceived threat and intensifies the stress response (Schwabe et al., 2008; Schwabe & Wolf, 2013). Also, loud auditory stimuli are commonly used in laboratory research to induce acute stress. This approach involves exposing participants to sudden or intense sounds, such as bursts of white noise, alarms, aversive tones, or unpredictable loud noises presented through headphones or speakers. Because the human auditory system is highly sensitive to abrupt acoustic changes, these stimuli rapidly activate the body's defensive systems. In particular, loud sounds trigger sympathetic nervous system activation, leading to immediate physiological responses. These reactions reflect activation of the SAM system, which prepares the organism for rapid behavioral responses to potential threats (Ulrich-Lai & Herman, 2009).

In a recent meta-analysis, Brunyé and colleagues (2025) reviewed 245 studies with around 700 reported effects to compare how different laboratory stress induction methods influence physiological stress responses. Overall, stress induction procedures produced moderate physiological responses, with stronger effects observed in the SAM system compared to the HPA axis. Importantly, the magnitude of these responses varied systematically depending on task characteristics. For example, socially evaluative stressors, such as the TSST, elicited stronger HPA axis activation, whereas physically demanding or cognitively demanding tasks tended to produce more immediate sympathetic activation. In addition, the timing of physiological measurements influenced the observed effects, with SAM responses peaking rapidly and HPA responses showing a delayed increase. Participant characteristics, such as sex, also modulated stress reactivity. These findings indicate that different laboratory stress tasks engage partially distinct physiological systems, underscoring the importance of selecting stress-induction methods appropriate to the specific research question (Brunyé et al., 2025).

Standardization is a key strength of these paradigms, ensuring that stress induction is consistent and replicable across studies and populations. Each protocol specifies clear parameters, such as task duration (usually between five and fifteen minutes), verbal instructions, and evaluative feedback, to reliably evoke threat and loss of control. Their validity is supported by converging evidence showing that stronger elements of social evaluation correspond to higher cortisol peaks and greater physiological arousal (Dickerson & Kemeny, 2004). Additionally, these paradigms are valuable for identifying individual differences in vulnerability, such as delayed autonomic recovery following stress exposure (Het et al., 2009). Stress induction is commonly verified through a multimodal assessment approach, combining biochemical and physiological measures. This often includes monitoring salivary cortisol, cardiovascular activity via ECG, and EDA. Successful induction is typically defined by measurable physiological shifts, for example, a heart rate increase exceeding 20%, a skin conductance rise greater than 1 µS, or a cortisol elevation of approximately 2–5 nmol/L within twenty minutes after the task (Dickerson & Kemeny, 2004; Kirschbaum et al., 1993).

Evidence suggests that both the Trier Social Stress Test (TSST), which is described in detail in the next section, and the MAST methods reliably activate physiological stress systems; however,

they may differ in the specific patterns of physiological activation they produce. In a recent experimental comparison, Raidl and colleagues (2026) reported that both TSST and MAST successfully elicited measurable stress responses across endocrine and autonomic systems. However, hormonal responses associated with activation of the HPA axis, particularly cortisol secretion, were largely comparable between the two paradigms. These findings suggest that the TSST may elicit stronger autonomic responses, whereas endocrine activation appears similar across the two stress-induction methods (Raidl et al., 2025). Among the paradigms discussed, the TSST is one of the most widely used for inducing psychosocial stress and was selected for the stress induction task in the study described in this thesis.

2.1.1 The Trier Social Stress Test (TSST)

The Trier Social Stress Test (TSST) is a standardized laboratory paradigm that reliably induces acute psychosocial stress by combining social-evaluative threat and uncontrollability, typically through public speaking and mental arithmetic performed under observation. In this context, mental arithmetic involves time-pressured serial calculations performed without external aids while participants receive evaluative feedback, thereby imposing cognitive load and reinforcing perceived lack of control. The TSST was developed because earlier laboratory stress protocols failed to reliably activate the HPA axis, prompting the creation of a socially evaluative stress paradigm capable of inducing robust endocrine and cardiovascular responses in most participants (Kirschbaum et al., 1993). A key feature of the TSST is the perception of being observed and evaluated by others, which increases pressure and concern about negative judgment. This social-evaluative component plays a crucial role in eliciting strong stress responses, as situations involving potential evaluation are known to activate both subjective and physiological stress systems.

The speech topic does not significantly affect cortisol responses, as alternative topics produce similar stress reactions to the traditional job interview task (Gu et al., 2022). The TSST-G (Von Dawans et al., 2011) adapts the paradigm for groups of three participants while preserving robust physiological stress responses. Zintel and collaborators (2025) showed that the TSST-G reliably induces both subjective and biological stress responses. In their study with 175 participants,

subjective stress, assessed using self-report measures, increased during the stress phases and decreased during recovery, while cortisol levels significantly increased, confirming activation of the stress system. The authors also observed differences between subjective and biological responses, highlighting the importance of measuring multiple indicators when studying stress. Virtual and augmented reality implementations (e.g. Zimmer et al., 2019) further extend the paradigm to immersive digital environments to control the environment more effectively.

For children, the TSST-C (Buske-Kirschbaum et al., 1997) replaces the job interview with age-appropriate tasks designed to elicit social-evaluative stress. For example, children are often asked to complete a storytelling task, such as inventing and narrating a story based on a picture or a short prompt, in front of unfamiliar evaluators who maintain a neutral or non-reinforcing expression. They may also receive subtle negative or corrective feedback (e.g., being told to speak more clearly or to continue when they hesitate), which heightens the sense of social evaluation and performance pressure. These elements, public speaking, social judgment, and lack of positive feedback, are critical for reliably activating the HPA axis in children, similarly to the adult TSST. More recent adaptations include videoconference-based protocols (Gunnar et al., 2021), which preserve the core elements of social evaluation while allowing remote administration. This combination of social evaluation and performance pressure consistently produces robust physiological and psychological stress responses providing measurable markers that are highly informative for studying stress mechanisms and evaluating potential interventions (Kirschbaum et al., 1993; Narvaez Linares et al., 2020).

The ecological validity of the TSST is demonstrated by its capacity to replicate situations involving social judgment and evaluation. This feature enhances the generalizability of findings across diverse populations, including both clinical and healthy individuals (Allen et al., 2014; Het et al., 2009). Moreover, some evidence suggests that stress responses elicited by the TSST are related to stress experiences in everyday life. For instance, (Lohaus et al., 2025) found that adolescents with higher baseline cortisol and subjective stress during the TSST reported greater stress symptomatology and higher levels of daily stress assessed through ecological momentary assessment (EMA). In addition, stronger cortisol increases during the TSST were associated with more somatic complaints in daily life. These findings suggest that laboratory-induced stress responses may reflect individual differences in real-world stress vulnerability.

Regarding the role of facial expressions in the TSST, Ringgold and colleagues (2025) investigated whether facial muscle movements during the TSST reflect physiological and psychological stress responses. Their findings showed that observable facial muscle activity was not strongly associated with physiological or self-reported stress responses. Although facial action units differed between the stress and control conditions, the expected grouping of these movements into common emotional categories such as anger, fear, or disgust was not supported. Moreover, individual action units did not significantly predict cortisol, salivary alpha-amylase, heart rate, or self-reported stress scores. The authors therefore concluded that facial expressions may not provide a straightforward or reliable indicator of acute psychosocial stress, and that their interpretation is likely highly context-dependent.

When an acute stress response is examined over time, it can be divided into four main phases: baseline reflecting the resting state, anticipation before the stressor, the immediate reaction during the stressor, and the recovery period afterward. These phases are well established in stress research. The allostatic load framework further explains that repeated or prolonged exposure to stress can disrupt this pattern, leading to abnormal responses such as exaggerated reactions or delayed recovery (Juster et al., 2010; McEwen, 1998).

The baseline phase reflects the resting state before any task-related instructions are provided. Participants remain in a quiet, eyes-open resting condition without specific cognitive demands, allowing the assessment of stress-related measures under homeostatic levels of heart rate and cortisol. This period provides an individualized reference state against which subsequent stress-induced deviations in physiological and neural activity can be interpreted (Vanhollebeke et al., 2022). In the anticipation or preparation phase, the stressor has not yet begun, but participants have already been informed about the upcoming evaluative speech task. Subjectively, this period is characterized by increasing anxiety, worry, and enhanced vigilance as individuals mentally prepare for speaking in public. Physiologically, it is typically accompanied by rising autonomic arousal and the initial mobilization of stress systems, reflected in early changes in heart rate and stress hormone release (Angioletti et al., 2025; Barzegar et al., 2023).

The acute phase, corresponding to the speech segment, represents the peak of psychosocial stress. During this period, participants are directly exposed to social evaluation and perceived judgment,

which intensifies feelings of pressure, performance anxiety, and heightened self-focused attention, characterized by increased monitoring of one's own behavior and concern about how one is perceived by others. Subjectively, the speech phase is marked by strong negative affect and a pronounced sense of being scrutinized. At the same time, physiologically it is accompanied by robust autonomic activation, including elevated heart rate and other stress-related bodily responses, indicating maximal engagement of the stress response systems (Vanhollebeke et al., 2022). The fourth phase is recovery, which begins immediately after the stressor ends. Unlike the anticipatory and reactive phases, the sources of threat, such as uncertainty, lack of control, and social evaluation, are no longer present. During this phase, the body and mind gradually return toward baseline, reversing the psychological and physiological changes triggered by stress (Vanhollebeke et al., 2022). The recovery time depends on the system being measured and can range from about 30 minutes to up to an hour, particularly for cortisol levels (Goodman et al., 2017). Understanding these temporal patterns is important because real-life stress rarely occurs as a single, isolated event, but rather unfolds as a process with distinct peaks and recovery phases (Hermans et al., 2014).

2.2 Psychological Questionnaires Assessing Traits Related to Stress Reactivity

In addition to physiological indicators, stress responses are shaped by stable psychological traits that influence how individuals perceive and react to stressors. Self-report questionnaires are widely used to assess these traits, including worry, anxiety sensitivity, emotion regulation, and interoceptive awareness. Rather than directly measuring stress itself, these instruments capture individual differences that may modulate stress reactivity and recovery (Campbell & Ehlert, 2012). An exception is the Perceived Stress Scale (PSS; Cohen et al., 1983), which assesses individuals' subjective appraisal of stress in daily life.

Several validated questionnaires are available to assess stress across different psychological dimensions, including:

- The Anxiety Sensitivity Index–3 (ASI-3; Taylor et al., 2007) measures anxiety sensitivity, defined as the tendency to interpret anxiety-related sensations as harmful or threatening. The ASI-3 includes three correlated dimensions, physical concerns, cognitive concerns, and social concerns, which together reflect a higher-order anxiety sensitivity factor. Studies have shown

that ASI-3 scores are positively associated with symptoms of anxiety, depression, and other forms of psychological distress, supporting its construct validity as a measure of vulnerability to anxiety disorders (Essau et al., 2010). Additionally, the scale demonstrates good internal consistency and convergent validity with measures of neuroticism and anxiety symptoms. Individuals with higher anxiety sensitivity tend to show amplified stress responses, as bodily sensations are more likely to be interpreted as threatening.

- Trait worry is typically assessed using the Penn State Worry Questionnaire (PSWQ; Meyer et al., 1990), one of the most widely used measures of generalized worry. The PSWQ captures the tendency toward excessive and uncontrollable worry, which is a central feature of generalized anxiety. Higher levels of worry are associated with prolonged anticipatory stress and slower recovery following stress exposure. Empirical studies show that PSWQ scores correlate significantly with measures of anxiety, stress, and depressive symptoms, indicating that worry is closely related to broader negative emotional states (Fresco et al., 2003; Meyer et al., 1990).
- Interoceptive sensibility can be assessed using the Body Perception Questionnaire (BPQ; Cabrera et al., 2018), which measures individuals' subjective awareness of bodily sensations such as heart rate, breathing, and gastrointestinal activity. Higher BPQ scores are often associated with increased anxiety sensitivity and heightened emotional reactivity, suggesting that individuals who report stronger bodily awareness may also show greater sensitivity to stress-related physiological signals (Cabrera et al., 2018). This heightened interoceptive awareness may lead to stronger subjective stress experiences during physiological arousal.
- General psychological distress is commonly measured using the Depression Anxiety Stress Scale – 21 items (DASS-21; Lovibond & Lovibond, 1995). This instrument provides three related subscales assessing symptoms of depression, anxiety, and stress. Research supports the convergent validity of the DASS-21, as its subscales show strong correlations with other established clinical measures such as the Beck Depression Inventory and the Beck Anxiety Inventory. These findings indicate that the DASS-21 reliably captures core dimensions of emotional distress. Higher levels of general distress are associated with increased vulnerability to stress and reduced resilience.
- One of the most widely used self-report measures of perceived stress is the Perceived Stress Scale (PSS; Cohen et al., 1983), which evaluates the degree to which individuals appraise their

lives as unpredictable, uncontrollable, and overloaded during the previous month. The PSS has demonstrated good reliability and validity across diverse populations and is commonly used in stress research to assess perceived stress levels. Unlike trait measures, the PSS reflects individuals' appraisal of stress in their daily lives and is more directly related to perceived stress levels.

- Emotion regulation strategies are frequently assessed using the Emotion Regulation Questionnaire (ERQ; Gross & John, 2003), which measures the habitual use of two major regulation strategies: cognitive reappraisal and expressive suppression. Cognitive reappraisal is generally associated with better emotional adjustment and lower levels of stress and negative affect, whereas expressive suppression tends to correlate with higher psychological distress and reduced well-being. Individuals who rely more on cognitive reappraisal tend to show more adaptive stress responses, whereas suppression is associated with increased physiological and subjective stress (Gross & John, 2003).
- The Intolerance of Uncertainty Scale (IUS; Carleton et al., 2007; Freeston et al., 1994), evaluates dispositional responses to uncertain situations. Individuals with high intolerance of uncertainty often experience greater worry, anxiety, and stress, and IUS scores have been consistently associated with measures of generalized anxiety and worry, particularly with instruments such as the PSWQ. High intolerance of uncertainty is linked to heightened anticipatory anxiety and exaggerated responses to unpredictable stressors.
- Finally, the State-Trait Anxiety Inventory – State version (STAI-S; Spielberger et al., 1983). The STAI-S is a widely used self-report instrument designed to measure transient anxiety, reflecting how individuals feel "at this moment." The scale consists of 20 items rated on a 4-point Likert scale, ranging from "not at all" to "very much so," with higher scores indicating greater levels of momentary anxiety. Unlike trait measures of anxiety, which capture relatively stable personality characteristics, the STAI-S is specifically intended to detect short-term fluctuations in anxiety in response to situational demands or stressors. The STAI has demonstrated high internal consistency and strong construct validity across both clinical and non-clinical populations, with reported Cronbach's alpha values typically above 0.90 (Spielberger et al., 1983). Previous research has also shown that STAI-S scores are positively correlated with other measures of psychological distress and stress-related symptoms, including anxiety and stress subscales of the DASS and measures of negative affect (

Spielberger et al., 1983; Julian, 2011; Lovibond & Lovibond, 1995). In experimental stress paradigms, the STAI-S is frequently used to assess changes in subjective anxiety across different stages of stress induction. Unlike the previously described trait measures, the STAI-S captures state-dependent fluctuations in anxiety and is therefore particularly suited to assessing momentary stress responses. Because of its sensitivity to short-term emotional changes, the STAI-S is considered a valuable subjective measure for complementing physiological indicators of stress, such as autonomic and neural responses.

2.3 Electroencephalography studies of stress

Electroencephalography (EEG) is a non-invasive technique that measures the brain's electrical activity via scalp electrodes, capturing voltage fluctuations generated by synchronized postsynaptic potentials in cortical neuronal populations (Schomer et al., 2011). EEG has become a fundamental methodology in stress neuroscience due to its unique ability to provide millisecond-level temporal resolution. However, these signals are spatially undefined due to volume conduction, meaning that electrical activity generated in one cortical region can spread through the brain, skull, and scalp and be detected at multiple electrodes. As a result, scalp topographies do not directly correspond to precise anatomical sources. Unlike other neuroimaging techniques such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET), which rely on slow hemodynamic or metabolic changes, EEG detects the brain's electrical dynamics in real time, making it exceptionally well-suited to capturing the fast, task-specific neural responses that characterize acute stress (Katmah et al., 2021). These features make EEG a practical and informative method for studying stress-related brain dynamics. Recent studies combining EEG with functional near-infrared spectroscopy (fNIRS) have shown enhanced spatial and temporal sensitivity for monitoring cortical stress responses (Fazli et al., 2012).

EEG is a well-tolerated and cost-effective technique that allows repeated measurements. Its portability makes it suitable for both laboratory and naturalistic settings, including wearable and wireless applications. EEG also integrates well with machine learning methods and multimodal physiological sensors, enabling real-time and adaptive analysis of brain–body states (Katmah et al., 2021). Acute stress reliably induces transient yet measurable changes in oscillatory activity

across multiple frequency bands, and these shifts are often spatially and temporally specific. One of the largest meta-analyses examining the neural correlates of psychosocial stress is the one by Vanhollebeke et al. (2022), which reviewed 34 studies. The results indicated that psychosocial stress reliably modulates EEG alpha activity, with a consistent reduction in alpha power, primarily over frontal electrode sites (Vanhollebeke et al., 2022). A widely accepted view in the EEG literature is that alpha power is inversely related to cortical activity, reflecting an inhibitory control mechanism of the brain (Allen et al., 2014; Jensen & Mazaheri, 2010b; Mathewson et al., 2011). Accordingly, reductions in alpha power during psychosocial stress can be interpreted as a consequence of increased arousal and vigilance, accompanied by activation of the HPA axis (Campbell & Ehlert, 2012; Kudielka et al., 2004). This shift toward lower alpha power reflects heightened cortical engagement, supporting adaptive cognitive and behavioral responses to the stressor.

Regarding beta power changes, the meta-analysis by Vanhollebeke and colleagues (2022) found no significant overall effects during the acute stress or recovery phases. However, several individual studies have reported increases in beta power during both acute stress exposure and the recovery phase (Al-Shargie et al., 2016; Perak, Malaysia et al., 2019; Guo et al., 2019; Perrin et al., 2019), However, another study reported a decrease in overall beta power when the beta band was subdivided into low (13–17 Hz) and high (18–30 Hz) frequencies, showing that only the low beta band exhibited a significant increase **(**Betti et al., 2018). Beta-band activity is commonly linked to active cognitive processing. Accordingly, increased beta power during the stress response can be interpreted as reflecting heightened ongoing cognitive engagement. This likely occurs because stress recruits additional neural circuits, enabling the individual to process demands more efficiently and adapt their behavior to psychosocial stressors (De Kloet et al., 2005b; McEwen, 2007). Beta-band oscillations have also been associated with top-down cognitive control processes that maintain task goals and direct attention. In contrast to bottom-up sensory processing, top-down modulation reflects the influence of higher-order cortical regions, particularly the PFC, on regulating perception and behavior in response to task demands.

Arsalan and Majid (2021) systematically evaluated the discriminative power of EEG frequency bands in distinguishing between rest and acute psychosocial stress induced by a public-speaking task. In their study, EEG signals were recorded during resting and stress conditions. Spectral

features were computed separately for each frequency band, including delta, theta, alpha, and beta, allowing the authors to directly compare the contribution of each band to stress classification. These features were then used as inputs to machine-learning classifiers, and classification performance was assessed using cross-validation to ensure robustness and generalizability across participants. Their analyses demonstrated that beta-band features consistently yielded the highest classification accuracy, outperforming delta, theta, and alpha bands when evaluated independently. Importantly, the superiority of beta activity was observed across validation folds, indicating that beta-band modulation provided stable and reliable information for distinguishing stressed from non-stressed states. The authors interpreted this finding as reflecting the sensitivity of beta oscillations to heightened cognitive engagement, alertness, and performance-monitoring demands associated with socially evaluative stressors, such as public speaking. Together, these results suggest that beta-band activity represents a particularly robust neural marker of acute psychosocial stress (Arsalan & Majid, 2021).

A study by Barzegar et al. (2023) investigated EEG changes during the acute phase of psychosocial stress using the TSST in 44 healthy male participants, with salivary cortisol measured to confirm successful stress induction. Cortisol levels increased significantly during the TSST, validating the effectiveness of the stress manipulation. EEG results revealed a shift toward lower-frequency activity during acute stress. Specifically, relative delta power (1–4 Hz) increased significantly, while beta-band activity (13–30 Hz) decreased markedly. Smaller reductions were also observed in the theta band and gamma-1 range (30–40 Hz), particularly over frontal regions. In addition to spectral changes, several nonlinear complexity measures, including approximate entropy, spectral entropy, and Katz fractal dimension, decreased during the TSST, resembling the pattern observed in the beta band. In the recovery phase following stress termination, most EEG measures returned to baseline levels. The only exception was a sustained increase in Katz fractal dimension at the left frontal site (F3), suggesting prolonged alterations in frontal cortical dynamics after stress exposure (Barzegar et al., 2023).

Beyond simple power measures, many studies also use power-derived EEG measures. These metrics assess changes in spectral power by combining information across spatially distinct electrode sites (e.g., frontal alpha asymmetry or the alpha–theta power ratio) or across different frequency bands (e.g., relative power measures). Among these approaches, frontal alpha

asymmetry (FAA) is the most widely used. FAA has been extensively studied in psychology and psychiatry and has been linked to a broad range of psychological processes as well as various psychiatric conditions (Smith et al., 2017). Analysis of the FAA revealed a significant modulation during the acute stress condition. Specifically, participants showed a relative reduction in alpha power over left frontal electrodes compared to right frontal sites, indicating increased left frontal hemispheric activity under stress. This leftward shift in FAA was evident during the stress phase compared to the control/baseline condition. No comparable asymmetry was observed outside the stress period, suggesting that the effect was state-dependent and specific to acute psychosocial stress exposure (Berretz et al., 2022).

Research on theta and gamma in the context of stress is still limited, and these bands are often not the primary focus of existing studies. Nevertheless, available evidence suggests that theta power is reduced during the acute phase of stress compared to both control and recovery conditions (Hafeez et al., 2018). Studies examining gamma-band activity typically report relative gamma, defined as gamma power normalized to total power across all frequency bands. During the acute phase, relative gamma was significantly higher in the stress condition compared to the control condition across all electrode locations. During the recovery phase, relative gamma decreased significantly compared to the reactive phase, again across all electrode sites (Minguillon et al., 2016, 2017).

Another EEG index that can be measured is the ratio between different frequency bands, which some studies have found to be meaningful. The theta–alpha power ratio reflects internal and external cognitive load and is defined as the ratio of theta power at the Fz electrode to alpha power at the Pz electrode (Holm et al., 2009). This measure was examined during the reactive and recovery phases of stress and was significantly higher in the stress condition compared to the control condition during the reactive phase. This difference did not persist during the recovery phase (Subhani et al., 2013). In emotionally charged stress contexts, especially during the anticipation or performance phases of the TSST, delta power (1–4 Hz) tends to rise**,** particularly in frontal and central regions. This low-frequency activity is believed to reflect motivational salience**,** emotional arousal**,** and internal attention allocation (Angioletti et al., 2025). The co-variation of delta and theta with heart rate and cortisol changes further supports their relevance as cross-domain biomarkers of stress reactivity (Gärtner et al., 2014).

Beyond frequency-domain analyses, stress-induced alterations in functional connectivity are gaining increasing attention. EEG-based connectivity measures such as coherence**,** phase-locking value (PLV)**,** and Granger causality have revealed decreased synchronization between prefrontal areas and posterior default mode network (DMN) hubs, including the posterior cingulate cortex (PCC)**.** These disruptions suggest that acute stress leads to the transient decoupling of introspective and regulatory networks**,** reallocating resources toward external threat vigilance (Vanhollebeke et al., 2022; Yan et al., 2024). Many EEG studies of stress have relied on aggregated pre–post comparisons, potentially obscuring task-specific dynamics. Task-resolved analysis allows for the identification of temporally distinct neural signatures associated with anticipation, exposure, and recovery (Vanhollebeke et al., 2022).

Individual differences are likely to shape responses to psychosocial stress, and recent work has suggested that stable trait factors such as personality may be reflected in stress-related EEG dynamics. In a TSST study using a 64-channel EEG, Riaz and colleagues (2025) proposed an EEG-based computational framework to classify the Big Five personality traits from spectral features across TSST phases using supervised machine learning. Their results indicated that higher scores in traits such as Extraversion, Conscientiousness, and Openness were associated with lower temporal theta-to-beta ratio (TBR), an EEG index reflecting the balance between slower (theta) and faster (beta) oscillatory activity. Lower TBR is generally interpreted as indicating greater cognitive control and engagement, which the authors associated with more efficient stress recovery and greater cognitive resilience during specific TSST phases (Riaz et al., 2025). Taken together, these findings support the idea that EEG during social stress may capture trait-like signatures relevant for understanding heterogeneity in stress reactivity and recovery, and they motivate considering personality as a potential moderator of brain-based stress markers in TSST research.

Recent multimodal research by Ahmed and colleagues (2025) comparing EEG, HRV, and EMG for stress detection found that although EEG achieved the highest accuracy when used alone, combining HRV and EMG signals produced classification performance comparable to EEG-based models. These findings suggest that wearable systems integrating autonomic and muscular measures could effectively detect stress without requiring direct brain recordings, highlighting the potential of peripheral physiological monitoring for scalable and ecologically valid stress assessment outside laboratory settings. (Ahmed et al., 2025)

2.4 Autonomic Psychophysiology Studies of Stress

This section reviews autonomic psychophysiological measures used to assess peripheral physiological responses during stress and the finding relative to this. In particular, it focuses on electrocardiography (ECG) and electrodermal activity (EDA), two widely used indicators of autonomic nervous system activation in response to stress. ECG-derived measures such as heart rate (HR) and heart rate variability (HRV) provide insight into cardiovascular activation and autonomic regulation during stress exposure and recovery. In contrast, EDA reflects sympathetic nervous system activity and is particularly sensitive to changes in physiological arousal during stressful situations. A large meta-analysis including 171 TSST studies and 8,452 healthy adults confirmed that acute psychosocial stress reliably elicits strong physiological responses, particularly within the autonomic nervous system (Gu et al., 2025). In this analysis, autonomic markers such as heart rate and blood pressure showed larger and more consistent effects compared with the HPA axis measures such as cortisol. Moreover, correlations between physiological and psychological stress responses were generally weak, suggesting that different stress systems may respond somewhat independently.

Peripheral stress responses can also be assessed through several physiological indicators beyond cardiovascular and electrodermal measures. One important measure is respiration, as stress often leads to changes in breathing patterns, including increased respiratory rate and reduced variability due to heightened sympathetic activation and increased metabolic demand. These respiratory changes are closely linked to autonomic regulation and emotional arousal, making respiration a useful marker of stress-related physiological activation (Homma & Masaoka, 2008). Another commonly used measure is pupillometry, which examines changes in pupil diameter. Pupil dilation is influenced by the autonomic nervous system and has been shown to increase during states of heightened cognitive load, emotional arousal, and stress (Bradley et al., 2008). In addition, electromyography (EMG) can be used to measure muscular activity, particularly in facial muscles involved in emotional expression. For example, increased activation of the corrugator supercilii muscle is often associated with negative emotional states and stress-related responses (Larsen et al., 2003). Finally, stress responses can also be assessed through salivary biomarkers, such as salivary alpha-amylase, which has been proposed as a non-invasive marker of sympathetic nervous system activation. Levels of salivary alpha-amylase typically increase rapidly during acute

psychosocial stress and are often used alongside cortisol to capture different components of the stress response (Nater & Rohleder, 2009).

Physiological stress responses do not always align across systems. Cardiovascular measures (e.g., heart rate, blood pressure) tend to correlate, whereas cortisol responses often show weak associations, suggesting partial dissociation between autonomic and endocrine systems (Guo et al., 2019). Age and sex may also influence stress reactivity, with older individuals showing stronger cardiovascular responses and some evidence of higher cortisol responses in men, although overall sex differences remain limited (Seeman et al., 2001).

2.4.1 Electrocardiography (ECG) in stress

ECG records the electrical activity of the heart and allows the derivation of heart rate (HR) and heart rate variability (HRV). HRV refers to the variation in the time intervals between consecutive normal-to-normal (NN) inter-beat intervals and represents the dynamic regulation of cardiac activity by the autonomic nervous system. HRV indexes the balance and flexibility of sympathetic and parasympathetic influences on the heart and is widely used as a physiological marker of autonomic regulation, stress reactivity, and recovery. (Appelhans & Luecken, 2006). While increases in the HR may reflect heightened sympathetic activation and metabolic demand during stress exposure, reductions in HRV, particularly in vagally mediated indices such as RMSSD and HF power, reflect parasympathetic withdrawal and reduced regulatory flexibility. Thus, HR and HRV provide complementary information: HR indexes the intensity of arousal, whereas HRV indexes the capacity for adaptive regulation and recovery (Appelhans & Luecken, 2006; Shaffer & Ginsberg, 2017). Examining both measures allows differentiation between stress activation and stress regulation.

There are different categories of HRV measures, each capturing distinct aspects of autonomic regulation. Time-domain measures focus on variability in interbeat intervals and provide important information about short-term autonomic control. The mean squared successive differences (MSSD) quantify the average of the squared differences between successive NN intervals and are sensitive to rapid fluctuations in heart rate. The root mean square of successive

differences (RMSSD) represents the square root of MSSD and is widely used because it preserves the original unit of measurement and is less influenced by outliers. Both MSSD and RMSSD primarily reflect parasympathetic (vagal) modulation of cardiac activity, as they capture high-frequency, short-term variability in heart rate (Shaffer & Ginsberg, 2017).

In addition to time-domain HRV indices, autonomic activity can be characterized using frequency-domain measures, including high-frequency (HF) power. HF power is widely interpreted as an index of parasympathetic (vagal) control of the heart and is known to be sensitive to stress-related changes and recovery processes (Laborde et al., 2017) . Including frequency-domain HRV measures allows for a more specific assessment of autonomic regulation and provides important information about task-specific parasympathetic withdrawal and recovery during psychosocial stress.

Studies employing various psychological stressors demonstrate that frequency-domain HRV metrics are modulated by stress, primarily indicating parasympathetic withdrawal. A review of 37 human studies on HRV reactivity to psychological stress published between 2007 and 2017 Kim and collaborators (2018) concluded that HRV is reliably reduced during stress, supporting its utility as an objective marker of stress-related autonomic regulation. This pattern is frequently accompanied by reductions in time-domain vagal indices, such as RMSSD, consistent with diminished parasympathetic control during stress exposure.

Importantly, autonomic responses to psychosocial stress are task-dependent. Anticipatory phases are often characterized by gradual parasympathetic withdrawal, whereas peak stress exposure is associated with marked increases in HR and reduced vagal indices. During recovery, HRV typically rebounds as parasympathetic activity is reinstated. However, delayed HRV recovery has been associated with reduced regulatory capacity and increased stress vulnerability (Campbell & Ehlert, 2012; Kim et al., 2018). Caution is warranted when interpreting the low-frequency (LF) band of heart rate variability. The LF band typically ranges from 0.04 to 0.15 Hz and reflects slower oscillations in heart rate associated with autonomic cardiovascular regulation. In earlier literature, LF power and the LF/HF ratio were often interpreted as indicators of sympathetic nervous system activity or “sympathovagal balance.” However, more recent evidence suggests that LF does not exclusively index sympathetic activation. Instead, LF power may reflect

a combination of sympathetic and parasympathetic influences, as well as baroreflex-mediated regulation of heart rate, depending on the experimental context and analytic approach. Consequently, increases in LF power or in the LF/HF ratio observed in some stress paradigms should be interpreted cautiously and within the broader pattern of HRV indices. This interpretation is consistent with meta-analytic findings from clinical anxiety research showing that LF changes cannot be attributed solely to sympathetic activation (Chalmers et al., 2014).

Substantial inter-individual variability exists in both baseline HRV and stress-induced HRV reactivity. Individuals with higher resting vagal tone often exhibit more adaptive stress profiles, characterized by efficient activation during exposure and faster recovery thereafter. Conversely, exaggerated, blunted, or delayed HRV responses may reflect dysregulation of autonomic control and have been associated with increased vulnerability to stress-related psychopathology (Chalmers et al., 2014; McEwen, 2007). Given the dense bidirectional connections between prefrontal regulatory regions and autonomic control centers in the brainstem and hypothalamus, HRV has been proposed as a peripheral index of central regulatory processes (Thayer & Lane, 2009). Variations in HRV may therefore covary with neural oscillatory dynamics during stress, providing a physiological window into brain–body coupling mechanisms (Ulrich-Lai & Herman, 2009).

2.4.2 Electrodermal activity (EDA) in stress

By contrast to ECG, EDA measures only sympathetic nervous system (SNS) activity, as eccrine sweat glands are innervated exclusively by sympathetic cholinergic fibers and receive no parasympathetic input. Consequently, changes in skin conductance directly reflect variations in sympathetic arousal without confounding parasympathetic influences (Boucsein, 2012).

Electrodermal activity can be decomposed into tonic and phasic components that reflect different aspects of sympathetic arousal. The tonic component represents the absolute level of skin resistance or conductance at a given moment in the absence of discrete event-related responses and is commonly referred to as skin resistance level (SRL) or skin conductance level (SCL). Superimposed on this tonic background are phasic changes, characterized by transient decreases

in skin resistance or increases in conductance, termed skin resistance responses (SRRs) or skin conductance responses (SCRs), typically induced by eliciting experimental stimuli.

Another commonly used phasic index of electrodermal activity is the number of spontaneous fluctuations (nSF), defined as the number of SCR peaks not specifically linked to any eliciting stimulus occurring within a given time window. This measure reflects the frequency of transient sympathetic activations and is therefore considered a useful indicator of overall sympathetic arousal. Higher nSF values indicate more frequent sympathetic bursts, typically observed during conditions of increased cognitive or emotional demand, including stress-inducing paradigms such as the Trier Social Stress Test (Boucsein, 2012; Dawson et al., 2007).

Under conditions of stress or anxiety, both tonic skin conductance level and phasic skin conductance responses typically increase, and these changes often covary with subjective reports of anxiety, cognitive load, and negative affect. Such effects have been consistently observed across a range of experimental tasks, including psychosocial stress paradigms (e.g., public speaking and social evaluation tasks such as the TSST), cognitively demanding tasks (e.g., mental arithmetic, Stroop, and working memory paradigms), emotional processing tasks involving negative or arousing stimuli, anticipatory and uncertainty-based threat paradigms, and pain or discomfort induction tasks (Boucsein, 2012; Dawson et al., 2007).

In the context of the TSST, combining EEG with ECG- and EDA-based measures enables a multimodal characterization of the stress response, linking central neural dynamics to peripheral autonomic activity. Within this framework, HR and HRV provide information about cardiovascular engagement, regulatory capacity, and sympathovagal balance, whereas EDA indexes the intensity and temporal profile of sympathetic arousal (Kim et al., 2018; Shaffer & Ginsberg, 2017).

Together, ECG and EDA provide important information about how the autonomic nervous system responds and adapts to psychosocial stress and complement EEG-based measures of central nervous system activity (Shaffer & Ginsberg, 2017). Further research by Lee et al. (2024) examined autonomic responses during a TSST-based stress protocol using both the HRV and the EDA. Their findings indicated that EDA parameters, particularly SCR, were more sensitive to

acute stress reactivity during stress exposure. In contrast, HRV measures showed greater changes during the recovery phase following stress, reflecting parasympathetic regulation of cardiovascular activity.

Another important distinction between ECG-derived cardiovascular measures and EDA concerns their temporal dynamics during stress exposure. Electrodermal responses typically show rapid phasic increases within a few seconds after emotionally salient or threatening stimuli, reflecting fast sympathetic activation of eccrine sweat glands. Cardiovascular responses, in contrast, often evolve over slightly longer time windows and reflect the integrated influence of sympathetic activation and parasympathetic withdrawal on cardiac regulation (Boucsein, 2012; Kreibig, 2010). In summary, ECG and EDA provide complementary insights into autonomic stress responses. Their combined use enables a more comprehensive assessment of cardiovascular regulation and sympathetic arousal during stress.

Chapter Three: Methods & Hypothesis

3.1 Study Rationale & Aim

Although psychosocial stress research has advanced considerably, important gaps remain in understanding its underlying mechanisms. Key limitations in prior studies include methodological and measurement fragmentation, inconsistent findings regarding neural indicators of stress, a predominant focus on peak stress responses rather than task-specific dynamics, limited consideration of individual differences, and insufficient investigation of interoceptive processes and mechanisms underlying brain–body associations in stress reactivity. Differences in methodological approaches across studies may influence the pattern of findings reported in the stress literature. For example, Campbell & Ehlert (2012), demonstrated that subjective stress reports do not consistently correspond to physiological activation, highlighting the limitations of relying solely on self-report measures. Furthermore, the meta-analysis by Vanhollebeke and colleagues (2022) documented substantial heterogeneity in EEG findings during the TSST, suggesting that differences in measurement approaches may influence reported outcomes. Together, these findings suggest that single-modality or isolated measures capture only part of the stress response. Therefore, integrating EEG with autonomic indices provides a more comprehensive characterization of stress dynamics by combining complementary information from central and peripheral systems (Arsalan & Majid, 2021).

When focusing only on acute stress, most studies using the TSST primarily compare pre- and post-stress conditions or average responses across participants and time windows. While this approach yields useful insights, it overlooks the dynamic nature of stress reactivity and adaptation as they unfold across time. The TSST comprises four psychologically distinct stages, each eliciting unique neurophysiological and stress levels. However, many TSST studies define stress reactivity as either the peak or task-averaged response relative to baseline, without systematically differentiating between anticipation, speech, and recovery phases (Kirschbaum et al., 1993). A further gap in the literature involves the heterogeneity of findings. The meta-analysis by Vanhollebeke and colleagues (2022), as discussed in Chapter 1, identified consistent effects exclusively in the alpha band, and no significant differences were reliably observed across studies

for beta-band activity or Frontal Alpha Asymmetry (FAA). Another instance of heterogeneity in the literature concerns high-frequency oscillatory activity. Some studies show beta power tends to increase during the reactive and recovery phases of psychosocial stress (Al-Shargie et al., 2016; Guo et al., 2019; Hafeez et al., 2018; Minguillon et al., 2016; Perrin et al., 2019). However, both Betti and colleagues (2018) and Ehrhardt and colleagues (2022) reported a decrease in beta power in response to a psychosocial stressor, although the effect was not significant. This variability is frequently attributed to methodological differences, variations in measurement timing, and inconsistent definitions of stress phases across studies (Barzegar et al., 2023; Salankar & Qaisar, 2023; Zhou et al., 2012). Collectively, these results indicate a need for additional research to clarify task-specific neural mechanisms underlying stress responses.

Many studies rely on group-level averages, obscuring important within-subject differences. Stress reactivity and recovery patterns can vary dramatically between individuals based on traits such as anxiety, coping style, or prior experience (Chida & Hamer, 2008). A within-subject, task-resolved approach can capture this variability, offering more accurate and personalized insights into the mechanisms of stress regulation (Kaldewaij et al., 2016).

The anticipation phase is particularly important in the stress response. It is often conceptualized as a passive waiting period preceding the stressor, yet, psychologically, it involves active and effortful processing (Gaab et al., 2005). During anticipation, individuals evaluate the upcoming situation, predict possible negative outcomes, and assess whether their coping resources are sufficient to meet the demands. According to the transactional model of stress (Folkman & Lazarus, 1985), stress responses arise from this cognitive appraisal process rather than from the event itself.

Despite this, many experimental studies have primarily focused on stress responses during or after exposure, often overlooking the dynamic processes unfolding across different phases of the stress experience. This highlights the need for approaches that can capture the temporal evolution of stress responses across anticipation, exposure, and recovery.

The aim of this thesis is to investigate the effects of psychosocial stress using a multimodal, task-resolved approach that integrates central and peripheral physiological measures**.** Unlike conventional pre/post paradigms, this approach aligns with recent calls in the literature for finer-grained neural monitoring of stress responses (Kreibig, 2010). By examining brain–body correlation over time, this work aims to move beyond static or group-averaged representations of stress and to capture interindividual variability in stress reactivity and recovery.

As discussed in Chapter 1, investigating physiological responses to acute stress is important not only for understanding the mechanisms of stress regulation but also for identifying potential health risks. Physiological responses to acute stress can provide valuable information about long-term health vulnerability. In particular, research has shown that individual differences in stress reactivity may predict future physical and psychological outcomes. Individuals who consistently display stronger cardiovascular, endocrine, or inflammatory responses during acute stress tasks are more likely to develop conditions such as hypertension, metabolic disturbances, and other stress-related disorders later in life (Steptoe et al., 2007; Turner et al., 2020). Therefore, examining brain and body responses to acute psychosocial stress may contribute to a better understanding of how stress-related physiological processes develop and how early markers of vulnerability might be identified.

3.2 Methodology

3.2.1 Participants

In total, 153 individuals were contacted for recruitment. Six participants completed a pilot session (4 females, 2 males), and 52 participants met eligibility criteria for the main study. After excluding datasets with technical failures and excessive EEG noise, the final sample consisted of 38 healthy adults (age: Mean = 21.97 years, SD = 2.55, Min=18, Max=27), with a mixed-sex composition (20 females, 17 males, and 1 non-binary participant). Participants were recruited via campus flyers, word of mouth, direct student recruitment, and announcements circulated via social media and messaging applications. Participation was voluntary and compensated with a €15 reimbursement. The study was approved by the Ethics Committee for Psychological Research (Area 17) of the

University of Padova (protocol number 940-b) and conducted in accordance with the Declaration of Helsinki and institutional ethical guidelines for psychological research.

3.2.2 Experimental design and procedure

Participants first completed an online screening questionnaire prior to laboratory enrollment. Eligible participants then took part in a three-day protocol that combined laboratory assessment with ambulatory monitoring in daily life. In addition to the three consecutive lab sessions conducted at the same time of day, participants were enrolled in an ecological momentary assessment (EMA) protocol delivered via smartphone, through which they completed repeated brief questionnaires on momentary mood, worry, anxious arousal, body awareness, and stress-related contextual factors in their everyday environment. Throughout the study, they also wore a Polar watch to continuously monitor heart-rate activity outside the laboratory, providing an index of real-world autonomic fluctuations across the three days. This broader design allowed the project to capture both controlled stress responses in the lab and stress-related processes in daily life; however, the present thesis focuses specifically on the laboratory-based measures collected during the Day 2 stress-exposure session.

On Day 1 (Baseline Session, T1), resting-state EEG and peripheral physiological measures (ECG and EDA) were collected, together with a heartbeat counting task (Schandry, 1981) and self-report questionnaires, to establish baseline neural and autonomic and introceptive activity. Participants were told that they would return to the lab on Day 2 to undergo a moderately intense stress induction On Day 2 (Stress Session, T2), participants underwent the Trier Social Stress Test (TSST), during which EEG, ECG, EDA, and repeated state anxiety measures were recorded across the different experimental phases. On Day 3 (Recovery Session, T3), a resting-state recording was acquired, including EEG and peripheral physiological measures (ECG and EDA), along with self-report questionnaires, to assess post-stress recovery patterns. The present thesis focuses specifically on the second session (T2), during which the TSST was administered and stress-related neural and physiological responses were examined. The other tasks and measures (T1, T3) will not be discussed further.

The laboratory session lasted approximately 45 minutes and was conducted in the Geodesic Lab at the Department of General Psychology. Upon arrival, participants provided written informed consent, were seated in a quiet room under standard lighting conditions, and answered questions about the number of hours they slept and a worry rating on a scale from 0 to 100 ("How much you are worried about the stress induction on a 0 to 100 scale?"). EEG, ECG, and EDA sensors were then applied according to standard preparation and montage procedures (see below). Participants were instructed to minimize movement and remain as still as possible during recordings. From the beginning of the session, participants were seated facing a centrally positioned video camera in the room, as shown in Figure 3. During baseline and preparation, the camera remained visibly present but switched off. Before the speech phase, participants were informed that the camera would be activated so that an external audience of evaluators could observe and judge their performance. This instruction was intended to enhance the social-evaluative threat component of the TSST and increase perceived uncontrollability. In reality, the camera was never recording, and no external evaluators were present.

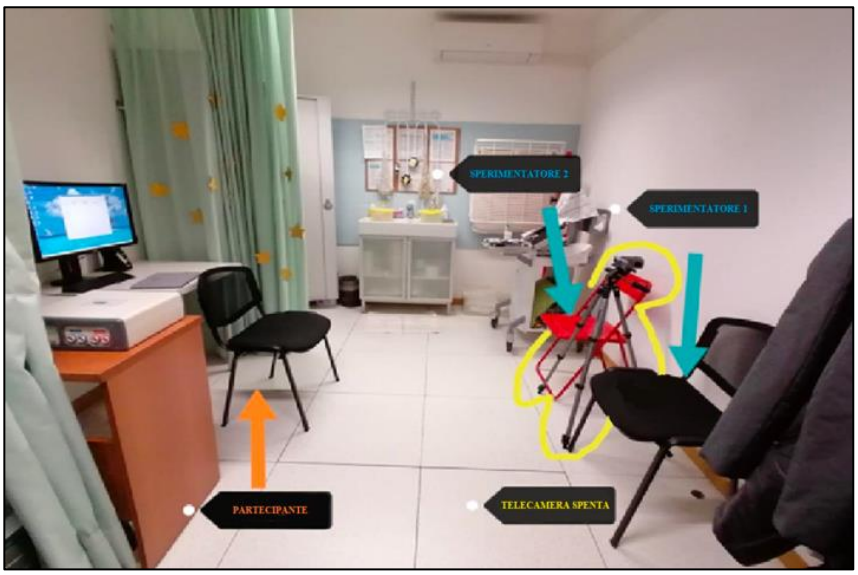


Figure 3. *Setting of the Lab for the second session T2*

The experimental protocol followed the typical TSST structure (Kirschbaum et al., 1993) and was implemented as a fixed within-subject sequence comprising four consecutive phases, as shown in

Figure 4. Each phase was structured to reflect a distinct stage of the stress response, enabling assessment of task-specific neural and autonomic activity, as described below.

1. Baseline (Resting State): Participants sat quietly for five minutes with their eyes open, Participants were instructed to remain as still as possible, to fixate on the cross, and to minimize excessive movements to reduce physiological artifacts. No specific cognitive or behavioral task was required. This condition served as an individual resting-state reference against which subsequent stress-related changes in neural and autonomic activity were compared.
2. Preparation (Anticipatory Stress): Participants were informed that they would deliver a job interview-style speech in front of an evaluator and a visible camera. They were instructed to imagine that they were applying for their ideal position and to convince the evaluator of their suitability. Participants were given five minutes to mentally prepare their speech without writing notes or receiving external assistance. The presence of the evaluator and the camera was emphasized to increase the task's social-evaluative threat.
3. Speech (Acute Stress): Participants delivered a five-minute speech while seated in front of a neutral, non-reactive experimenter and a prominently positioned video camera. The evaluator maintained a neutral facial expression, avoided verbal encouragement or corrective feedback, and intermittently took notes to reinforce the impression of a formal evaluation. If participants stopped speaking before the five-minute interval had elapsed, standardized prompts were used to encourage continuation. The experimenter interrupted the speech after 5 minutes, regardless of whether the participant had completed their presentation.
4. Recovery: Following the speech, The evaluator was no longer present in front of the participant. No task instructions were provided, and participants were asked to sit quietly with eyes open while minimizing movement. This five-minute phase allowed for the assessment of early post-stress recovery dynamics under non-evaluative conditions.

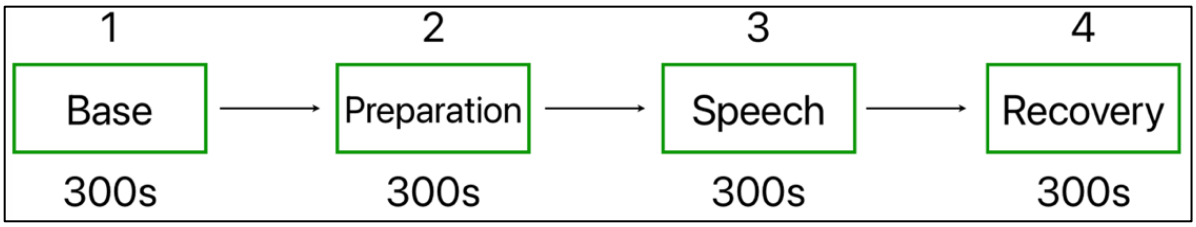


Figure 4. *TSST procedure*

Immediately after the TSST, participants were fully debriefed regarding the simulated nature of the evaluation. They were informed that the camera had remained off and that no external audience had observed their performance. If signs of significant emotional distress emerged during the procedure, clarification about the fictitious evaluation was provided immediately, in accordance with the ethical protocol.

3.3 Measures

3.3.1 Questionnaire measures

Before attending the laboratory sessions, all participants completed an online screening questionnaire via Qualtrics. This survey collected information regarding inclusion and exclusion criteria, as well as relevant self-report measures. Participants who met the eligibility requirements were subsequently scheduled for the laboratory sessions. Exclusion criteria included a history of neurological, psychiatric, or cardiovascular disorders; current use of psychoactive substances; and uncorrected visual or auditory impairments. Only individuals with normal or corrected-to-normal vision and hearing were included in the study.

Several self-report questionnaires were administered to characterize participants' psychological traits related to stress, anxiety, and emotional regulation. These included the Anxiety Sensitivity Index–3 (ASI-3; Taylor et al., 2007), the Penn State Worry Questionnaire (PSWQ; Meyer et al., 1990), the Body Perception Questionnaire (BPQ; Cabrera et al., 2018), the Depression Anxiety Stress Scales – 21 items (DASS-21; Lovibond & Lovibond, 1995), the Emotion Regulation

Questionnaire (ERQ; Gross & John, 2003), and the Intolerance of Uncertainty Scale (IUS; Freeston et al., 1994; Carleton et al., 2007).

Although these questionnaires were collected to characterize individual differences, they were not included in the analyses of the present thesis. The current analyses focused exclusively on state anxiety, measured using the State-Trait Anxiety Inventory – State version (STAI-S; Spielberger et al., 1983). The full questionnaire is available in Appendix 2. The STAI-S is particularly suitable for experimental stress paradigms because it captures rapid, transient fluctuations in anxiety that occur over short time periods in response to situational stressors. In the present study, the STAI-S was administered at each phase of the TSST (baseline, preparation, speech, and recovery) to capture phase-specific changes in subjective anxiety. STAI-S scores were visually inspected through plots to assess task-related changes in subjective stress across the experimental procedure, with higher scores expected during the acute stress phases.

3.3.2 EEG recording

EEG recordings were conducted in a quiet laboratory room with participants seated comfortably to minimize movement-related artifacts. EEG was continuously recorded across all TSST phases (baseline, preparation, speech, recovery) using a Geodesic high-density EEG System (EGI® GES-300) with a precabled 128-channel HydroCel Geodesic Sensor Net (HCGSN-128), detailed montage procedure included in Appendix 1. All electrodes were referenced online to the vertex (Cz) which is standard in high-density EEG systems and allows stable signal acquisition across electrodes. Scalp voltages were amplified through a 24-bit DC amplifier. The sampling rate was 500 Hz. The impedance was kept below 60 kΩ for each sensor, which is consistent with recommendations for high-impedance EGI systems. EEG recordings were preprocessed using the EEGLAB toolbox in MATLAB.

The preprocessing pipeline consisted of several sequential steps. Continuous data were first downsampled to 250 Hz. Line noise at 50 Hz was removed using the Zapline plugin (De Cheveigné, 2020), and the signal was subsequently band-pass filtered between 1 and 45 Hz. Artifact correction was performed using Independent Component Analysis (ICA; Stone, 2002) using the Infomax algorithm (Bell & Sejnowski, 1995). All independent components were visually

inspected, and those reflecting eye blinks, eye movements, cardiac activity, or muscle artifacts were identified based on their temporal characteristics and scalp topography and removed. The remaining components were then projected back into the electrode space. The cleaned continuous data were segmented into 2-second epochs for further artifact rejection. An automatic rejection procedure was applied on an epoch-by-epoch basis: channels exceeding a differential average amplitude of ±100 μV in more than 30% of epochs were marked as bad and interpolated using spherical spline interpolation (Ferree, 2006; F. Perrin et al., 1989). Epochs containing more than 10 bad channels were excluded from subsequent analyses. Finally, the data were re-referenced to the common average reference. Data from 10 participants were discarded at the end of the pre-processing due to a noisy EEG signal: epochs removed for each condition exceeded the a priori defined threshold of 30% (i.e., 50 epochs).

As expected due to speech artifacts on the EEG signal, the mean number of accepted epochs differed across experimental phases: baseline (M = 148.88, SD = 4.05), preparation (M = 146.69, SD = 5.90), speech (M = 118.77, SD = 28.63), and recovery (M = 143.54, SD = 14.53). Power spectral density (PSD) was estimated for each phase of the TSST (baseline, preparation, speech, and recovery) using Welch's method (Welch, 1967), as implemented in EEGLAB. This approach estimates spectral power by dividing the cleaned EEG signal into overlapping time windows, computing the Fast Fourier Transform (FFT) for each segment, and averaging the resulting periodograms to obtain a stable estimate of the power spectrum. PSD was calculated within the canonical frequency bands (delta: 1–4 Hz; theta: 4–8 Hz; alpha: 8–13 Hz; beta: 13–30 Hz; gamma: 30–45 Hz) and expressed in $\mu V^2/Hz$.

In this thesis, we focus mainly on the alpha and beta bands, which have been linked to cortical engagement and arousal-related processing (Engel & Fries, 2010; Klimesch, 2012). For each participant and condition, spectral power was averaged across artifact-free epochs and then aggregated within predefined electrode clusters based on scalp topography into 4 clusters (Frontal, Left Central, Right Central, Occipital), as illustrated in Figure 5. This clustering approach reduces dimensionality and improves signal reliability by grouping electrodes based on their anatomical proximity and functional relevance.

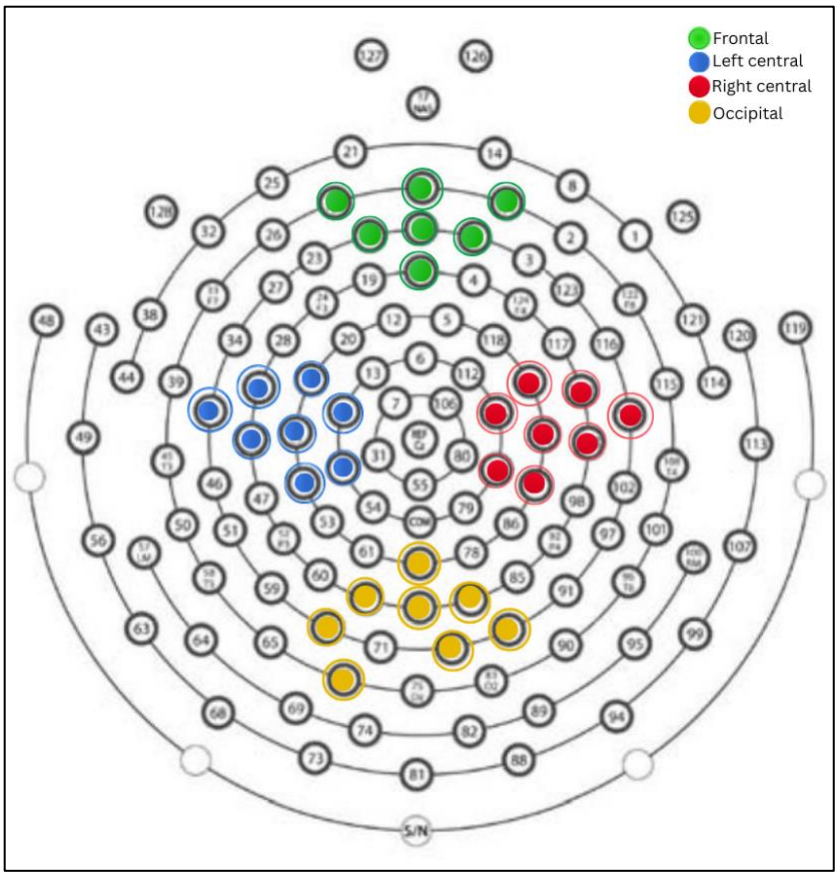


Figure 5. *Topographical Representation of Predefined Electrode Clusters Using the 128-Channel HydroCel Geodesic Sensor Net (HCGSN-128)*

The clusters included: frontal (E9, E10, E11, E15, E16, E18, E22), right central (E87, E93, E103, E104, E105, E109, E110, E111), left central (E29, E30, E35, E36, E37, E40, E41, E42), and occipital (E62, E66, E67, E70, E72, E76, E77, E84). Cluster-level PSD was computed as the mean power across electrodes within each region. Finally, PSD values were natural log-transformed to normalize the distribution prior to statistical analyses.

3.3.3 ECG recording

ECG was continuously recorded during all TSST phases (baseline, preparation, speech, recovery) using the PLUX Biosignals ECG sensor (PLUX Biosignals, Lisbon, Portugal). Before the recording began, participants were briefly shown the physiological recording equipment and the placement of the sensors to familiarize them with the procedure. Before electrode placement, the skin was cleaned to improve electrode–skin contact and ensure stable signal acquisition. The

detailed procedure is described in Appendix 1. Three Ag/AgCl electrodes were placed on the participant's chest and lower torso in a modified Lead II configuration, as illustrated in Figure 6. Disposable surface Ag/AgCl electrodes were used for each participant and discarded after the recording session to ensure hygiene and signal reliability.

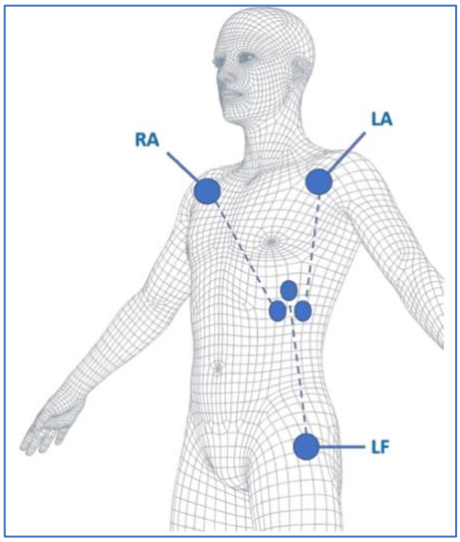


Figure 6. *Electrode placements for ECG acquisitions*

ECG raw data were processed in MATLAB using a combination of automated and manual procedures, following established guidelines for heart rate variability analysis (Laborde et al., 2017). Data were analyzed using Kubios HRV Analysis Software 2.0 (Tarvainen et al., 2014). Data were visually inspected, and ectopic and wrongly detected peaks were manually corrected. Time intervals between successive R-peaks (RR, msec) were computed to derive the individual average HR (bpm) across the TSST phases.

3.3.4 EDA recording

SC was continuously recorded across all TSST phases (baseline, preparation, speech, recovery) using the PLUX EDA sensor (PLUX Biosignals, Lisbon, Portugal). Before the recording started, the device was shown to the participant, and the procedure was briefly explained. Prior to electrode placement, the skin was gently washed with neutral soap and tempered water to ensure good electrode–skin contact. The detailed procedure is described in Appendix 1. Two surface Ag/AgCl

electrodes were attached to the non-dominant hand, positioned on the middle phalanx of the index and middle fingers. SC raw data were processed in Ledalab by downsampling to 10 Hz and applying an 8-point Gaussian smoothing. Artifacts were visually identified and corrected using a spline interpolation. A continuous decomposition analysis (CDA) was run using the "optimize" function implemented in Ledalab (Benedek & Kaernbach, 2010). From the CDA, we extracted the skin conductance level (SCL, the mean EDA value within the analyzed window, μS), the number of skin conductance responses (nSCR), and the mean amplitude of skin conductance responses (SCR, μS).

3.4 Hypothesis

This thesis aims to address two main research questions**.** The first question is whether stress induction is associated with differences in canonical EEG activity (delta, theta, alpha, and beta) across task phases (baseline, anticipation, speech, recovery) and regional scalp clusters (frontal, left, and right central, occipital). Second, the thesis explores individual differences in stress reactivity, assessing whether variability in brain activity is significantly associated with individual differences in sympathetic physiological responses.

3.4.1 HP1: Stress-phase & cluster effects on EEG power

Psychosocial stress induced by the TSST is expected to elicit significant changes in neural and peripheral physiological activity across experimental phases. PSD in canonical EEG frequency bands is therefore expected to differ across the four phases of the TSST. Specifically, the speech phase is hypothesized to produce the strongest neural and autonomic activation, while the baseline phase is expected to reflect minimal stress-related activity, with intermediate responses during anticipation and recovery. More specifically:

- A main effect of phase on the PSD is expected, indicating significant differences in oscillatory power across the baseline, preparation, speech, and recovery phases.
- During the acute stress (speech) phase relative to baseline, alpha-band PSD is expected to decrease, whereas beta-band PSD is expected to increase.

- Regional differences in EEG power across electrode clusters (frontal, left central, right central, occipital) are expected. In addition, we explore whether phase-related modulation of PSD varies across regions (phase × cluster interaction), with greater PSD in frontal and occipital clusters than in central clusters.

3.4.2 HP2: Brain–body correlation & individual differences

Beyond task-dependent effects, we expected meaningful individual differences in how neural activity relates to peripheral and subjective stress responses. We hypothesized that differences in brain activity during the speech phase would be associated with the strength of individuals' stress responses at both physiological and subjective levels. Based on research linking cortical activity to autonomic stress regulation (Thayer & Lane, 2000) and evidence indicating that acute stress modulates oscillatory activity (Vanhollebeke et al., 2022), we hypothesized that individuals with stronger physiological and subjective stress responses would show greater changes in cortical oscillatory activity during the speech phase. Specifically, alpha-band power is inversely related to cortical activation, such that reductions in alpha power reflect increased attentional engagement and neural activation (Klimesch, 2012). Accordingly, we expected that greater alpha suppression from baseline to speech would be associated with stronger sympathetic activation and higher self-reported anxiety. Beta-band activity has been associated with increased task engagement and heightened arousal (Engel & Fries, 2010) and has been reported to change during acute psychosocial stress, although findings remain heterogeneous (Vanhollebeke et al., 2022). Therefore, we anticipated that increases in beta power from baseline to speech would be positively associated with sympathetic reactivity.

Chapter Four: Analyses & discussion

4.1 Analyses

4.1.1 Stress Reactivity (Δ Scores)

For clarification of the subsequent analyses, stress reactivity in this thesis is operationalized as the change from baseline in central and peripheral measures during the TSST. Baseline was treated as a reference condition representing a relatively low-arousal state. Phase-dependent effects across baseline, preparation, speech, and recovery were examined using linear mixed-effects models (LMM) to capture within-subject changes over time. For between-subject analyses (HP2), delta (Δ) scores were computed relative to baseline, using the speech phase as the peak stress condition. Specifically, positive delta values indicate greater activation during speech, and negative values indicate greater activation during baseline. Δ scores were calculated for EEG power for both frequency band (Alpha, Beta), as well as for autonomic measures (HR, SCL, SCR, nSCR) and subjective stress (STAI-State).

4.1.2 HP1 Analysis: Stress-phase and cluster

To test the first hypothesis, we fitted separate linear mixed-effects models (LMMs) for each frequency band (delta, theta, alpha, beta). Phase (baseline, preparation, speech, recovery), electrode cluster (frontal, right central, left central, occipital), and their interaction were included as fixed effects, and a random intercept for participant was added to account for inter-individual variability; all the detailed statistical tables are available in Appendix 3. LMMs are well-suited for repeated-measures EEG designs and are robust to unequal numbers of accepted epochs across phases. Model significance was evaluated using F-tests with degrees of freedom approximated via Satterthwaite's method ($\alpha = .05$; R package: *lmerTest*; Kuznetsova et al., 2017). Post hoc pairwise comparisons were conducted using estimated marginal means contrasts (R package *emmeans*; Lenth, 2016).

4.1.3 HP2 Analysis: Individual differences

To test the second hypothesis, we tested whether EEG measures were associated with autonomic and subjective stress reactivity. To this aim, we conducted two sets of pairwise Pearson correlation analyses. First, we computed correlations between absolute PSD power in each canonical frequency band (delta, theta, alpha, beta) during the speech phase and Δ (delta) scores for peripheral and subjective measures (ΔHR, ΔSCL, ΔSCR, ΔnSCR, ΔSTAI-State). This analysis allowed us to assess whether neural activity at the peak stress phase was directly associated with the magnitude of sympathetic, cardiovascular, and subjective responses. Second, we computed Pearson correlations between delta (Δ) EEG power values and the corresponding delta (Δ) peripheral and subjective measures. This approach focuses on dynamic neural change induced from baseline to stress rather than on absolute power levels. By subtracting baseline activity from speech activity, this contrast isolates acute stress reactivity while controlling for tonic inter-individual differences in resting EEG power. All correlations were computed separately for each frequency band. Pearson's r was used as a measure of linear association between variables. Statistical significance was evaluated at $\alpha = .05$. Correlation analyses were exploratory and not corrected for multiple comparisons; therefore, the results should be interpreted cautiously. This analytical strategy allows us to determine whether variability in brain oscillatory dynamics reflects meaningful differences in the strength of individuals' responses to psychosocial stress at the physiological and experiential levels.

4.2 Result

4.2.1 Result of Manipulation check (descriptive)

Visual inspection of the boxplots in Figure 7 and the descriptive statistics in Table 1 suggests an increase in heart rate, electrodermal activity indices (SCL and nSCR), and STAI-State scores from baseline to preparation, with the highest values generally observed during the speech phase. More detailed statistical information is provided in Appendix 4. Following the end of the speech phase, these measures tended to decrease during the recovery phase, although recovery levels varied

across participants and, for some measures, remained elevated relative to baseline. Importantly, these observations are based on descriptive trends in the plotted distributions rather than inferential statistical testing. The purpose of this section is therefore to provide a qualitative check that the task elicited a pattern consistent with increased arousal and perceived stress during the stress phases, which supports the validity of the subsequent hypothesis-driven analyses.

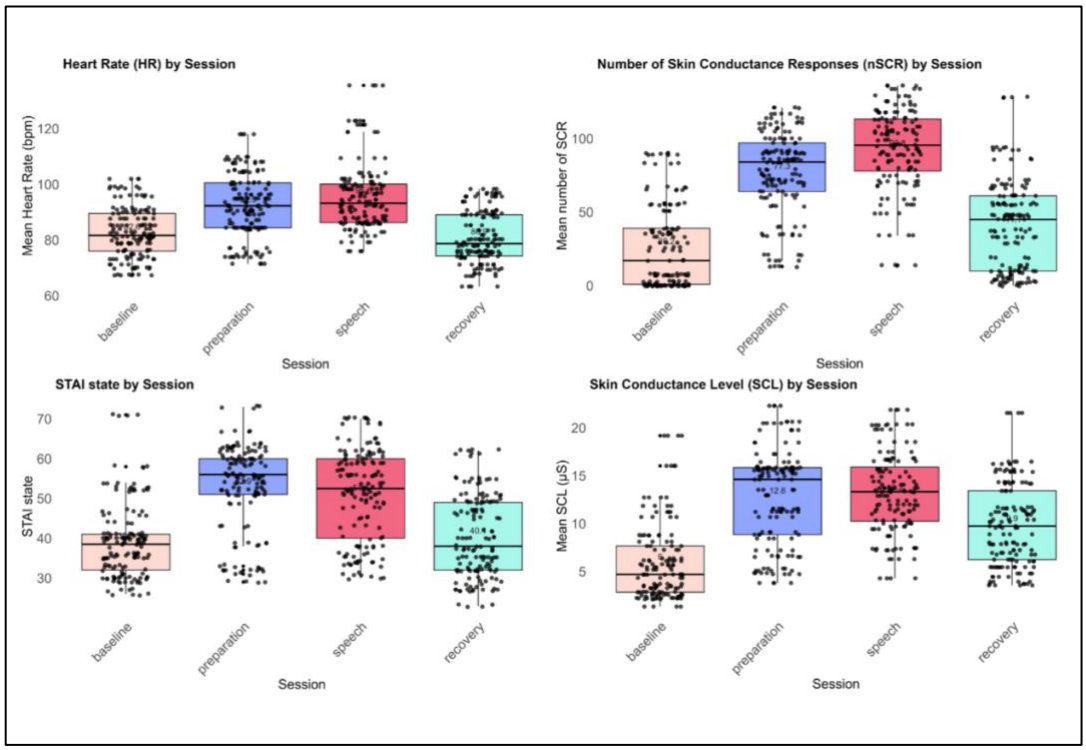


Figure 7. *Boxplot Phase-Dependent Changes in Physiological and Subjective Stress Measures Across the TSST,* Boxplots display mean heart rate (HR; beats per minute), number of skin conductance responses (nSCR), skin conductance level (SCL; µS), and state anxiety scores measured using the State version of the State–Trait Anxiety Inventory (STAI-S) across baseline, preparation, speech, and recovery phases. Each dot represents an individual participant.

Descriptive Statistics by Phase (Means)

phase	meanHR	meanSCL	meanSCR	nSCR	STAI_state
base	84.65520	6.868668	0.1807700	33.44828	40.44828
prep	93.28763	13.050268	0.3239570	78.94444	52.69444
spee	96.00256	13.174060	0.3039326	92.80556	51.02778
reco	80.85043	10.211950	0.2067791	43.34286	39.80000

Table 1. *mean values of Phase-Dependent Changes in Physiological and Subjective Stress Measures Across the TSST*

4.2.2 Result of HP1: Alpha band phase & cluster effects

A linear mixed-effects model revealed a significant main effect of cluster on alpha-band PSD, $F(3, 9) = 135.38$, $p < .001$ (see Table 1). To further examine this effect, post hoc pairwise comparisons were conducted. These analyses indicated that alpha-band PSD was significantly higher in the frontal cluster compared to both the left central cluster (estimated difference = 0.63, SE = 0.05, $t(555) = 11.51$, $p < .001$) and the right central cluster (estimated difference = 0.56, SE = 0.05, $t(555) = 10.32$, $p < .001$). In addition, alpha-band PSD was significantly higher in the occipital cluster compared to the frontal cluster (estimated difference = 0.30, SE = 0.05, $t(555) = 5.53$, $p < .001$), the right central cluster (estimated difference = 0.86, SE = 0.05, $t(555) = 15.85$, $p < .001$), and the left central cluster (estimated difference = 0.93, SE = 0.05, $t(555) = 17.04$, $p < .001$).

A significant main effect of phase on alpha-band PSD was also observed, $F(3, 9) = 13.88$, $p < .001$ (see Table 2). Post hoc pairwise comparisons were conducted to further explore differences across phases. These results showed that alpha-band PSD was significantly higher during baseline compared to preparation (estimated difference = 0.17, SE = 0.05, $t(555) = 3.16$, $p = .003$) and speech (estimated difference = 0.12, SE = 0.05, $t(555) = 2.27$, $p = .028$). In contrast, alpha-band PSD was significantly lower during baseline compared to recovery (estimated difference = −0.15, SE = 0.05, $t(555) = -2.74$, $p = .010$). Furthermore, alpha-band PSD was significantly lower during preparation compared to recovery (estimated difference = −0.32, SE = 0.05, $t(555) = -5.90$, $p <$

.001) and during speech compared to recovery (estimated difference = −0.27, SE = 0.05, $t(555) = -5.01$, $p < .001$).

Pairwise Contrasts ($contrasts)

contrast	estimate	SE	df	t.ratio	p.value
Scalp clusters (alpha-band PSD)					
front - centr_l	0.6272	0.0545	555	11.508	<.0001
front - centr_r	0.5623	0.0545	555	10.317	<.0001
front - occ	-0.3014	0.0545	555	-5.530	<.0001
centr_l - centr_r	-0.0649	0.0545	555	-1.191	0.2340
centr_l - occ	-0.9286	0.0545	555	-17.038	<.0001
centr_r - occ	-0.8637	0.0545	555	-15.847	<.0001
TSST phases (alpha-band PSD)					
base - prep	0.1721	0.0545	555	3.159	0.0033
base - spee	0.1237	0.0545	555	2.269	0.0283
base - reco	-0.1493	0.0545	555	-2.739	0.0095
prep - spee	-0.0485	0.0545	555	-0.889	0.3743
prep - reco	-0.3214	0.0545	555	-5.897	<.0001
spee - reco	-0.2730	0.0545	555	-5.008	<.0001

Table 1. *Pairwise comparisons between scalp clusters and TSST phases for alpha-band PSD.*

In summary, the combined effects of cluster and phase for alpha are illustrated in Figure 8. The figure shows a clear cluster pattern of alpha activity, with higher power observed over the frontal and occipital clusters. In addition, a noticeable suppression of alpha power occurs during the speech phase of the TSST and then relatively returns to baseline during recovery.

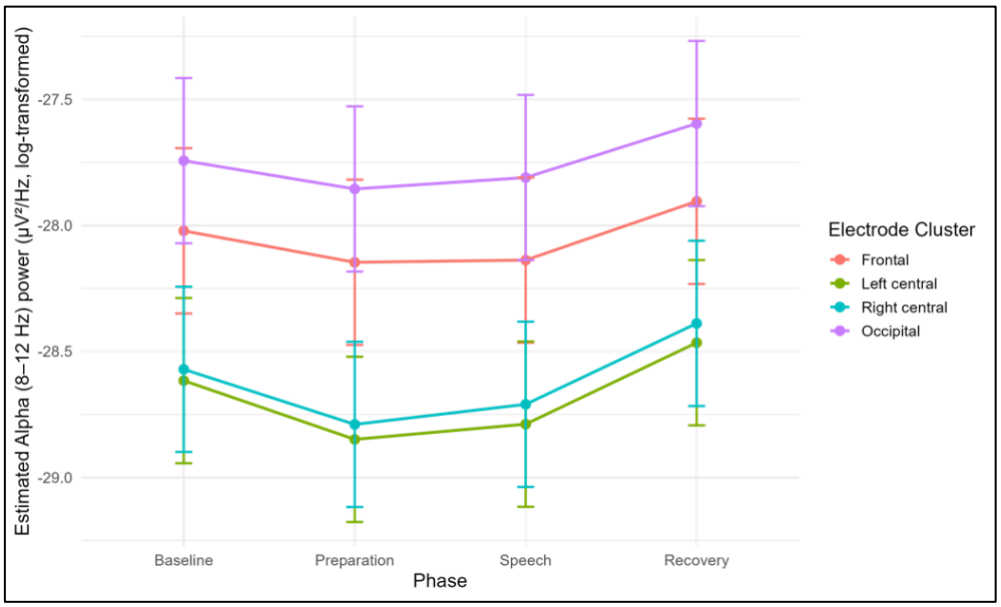


Figure 8. *Phase-Specific Modulation of Alpha-Band Power Spectral Density Across Scalp Clusters During the TSST*, Mean alpha-band power spectral density (PSD; ± SE) is shown for baseline, preparation, speech, and recovery phases across frontal, left central, right central, and occipital electrode clusters.

4.2.3 Result of HP1: Beta band phase & cluster effects

A linear mixed-effects model revealed a significant main effect of cluster on beta-band PSD, $F(3, 9) = 83.39$, $p < .001$ (see Table 3). To further examine this effect, post hoc pairwise comparisons were conducted. These analyses indicated that beta-band PSD was significantly higher in the frontal cluster compared to both the left central cluster (estimated difference = 0.49, SE = 0.05, $t(555) = 9.90$, $p < .001$) and the right central cluster (estimated difference = 0.43, SE = 0.05, $t(555) = 8.77$, $p < .001$). In addition, beta-band PSD was significantly higher in the occipital cluster compared to the frontal cluster (estimated difference = 0.16, SE = 0.05, $t(555) = 3.18$, $p = .002$), the right central cluster (estimated difference = 0.59, SE = 0.05, $t(555) = 11.95$, $p < .001$), and the left central cluster (estimated difference = 0.65, SE = 0.05, $t(555) = 13.08$, $p < .001$).

A significant main effect of phase on beta-band PSD was also observed, $F(3, 9) = 92.88$, $p < .001$ (see Table 3). Post hoc pairwise comparisons were conducted to further explore differences across phases. These results showed that beta-band PSD was significantly lower during baseline compared to preparation (estimated difference = −0.22, SE = 0.05, $t(555) = -4.34$, $p < .001$), speech (estimated difference = −0.80, SE = 0.05, $t(555) = -16.13$, $p < .001$), and recovery (estimated difference = −0.32, SE = 0.05, $t(555) = -6.48$, $p < .001$). Furthermore, beta-band PSD was significantly lower during preparation compared to both speech (estimated difference = −0.58, SE = 0.05, $t(555) = -11.78$, $p < .001$) and recovery (estimated difference = −0.11, SE = 0.05, $t(555) = -2.14$, $p = .033$). Finally, beta-band PSD was significantly higher during speech compared to recovery (estimated difference = 0.48, SE = 0.05, $t(555) = 9.64$, $p < .001$).

Pairwise Contrasts ($contrasts)

contrast	estimate	SE	df	t.ratio	p.value
Scalp clusters (Beta-band PSD)					
front - centr_l	0.489	0.0494	555	9.903	<.0001
front - centr_r	0.433	0.0494	555	8.769	<.0001
front - occ	-0.157	0.0494	555	-3.180	0.0019
centr_l - centr_r	-0.056	0.0494	555	-1.134	0.2572
centr_l - occ	-0.646	0.0494	555	-13.083	<.0001
centr_r - occ	-0.590	0.0494	555	-11.949	<.0001
TSST phases (Beta-band PSD)					
base - prep	-0.215	0.0494	555	-4.342	<.0001
base - spee	-0.797	0.0494	555	-16.125	<.0001
base - reco	-0.320	0.0494	555	-6.483	<.0001
prep - spee	-0.582	0.0494	555	-11.783	<.0001
prep - reco	-0.106	0.0494	555	-2.141	0.0327
spee - reco	0.476	0.0494	555	9.642	<.0001

Table 3. *Pairwise comparisons between scalp clusters and TSST phases for Beta-band PSD.*

In summary, as shown in Figures 8 and 9, the results supporting the first hypothesis revealed significant main effects of both phase and cluster across models, with no significant phase × cluster interactions. These results suggest that both phase and spatial cluster independently influenced alpha-band PSD during the TSST (see Appendix 5 for boxplot visualization). In the beta band, PSD increased progressively from baseline to preparation and from preparation to speech, then decreased during recovery. In contrast, alpha power showed an inverse profile, with higher PSD during baseline and recovery than during preparation and speech. This pattern is consistent with alpha suppression during anticipatory and active stress phases, followed by a rebound during recovery.

Across frequency bands, PSD was significantly greater in the frontal and occipital clusters relative to the central clusters, indicating a consistent scalp topographical pattern.

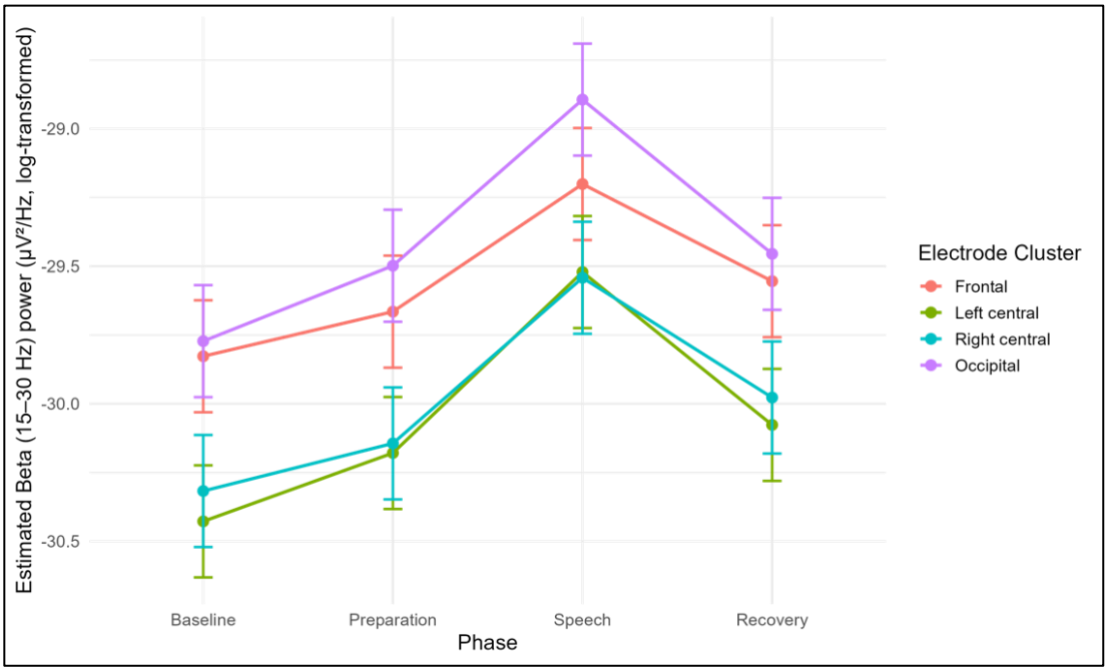


Figure 9. *Phase-specific modulation of beta-band PSD across scalp clusters during the TSST.*

4.2.4 Result of HP2

For the second hypothesis, as shown in Figure 10, we observed a significant negative correlation between alpha power during the speech phase and the change in the number of skin conductance responses ΔnSCR ($r = -.35$, $p = .035$). In addition, a significant positive correlation emerged between the change in beta power and ΔnSCR ($r = .34$, $p = .042$). These findings indicate that lower alpha power during speech was associated with a greater increase in nSCR from baseline to the speech period. Moreover, larger increases in beta power from baseline to speech were associated with greater increases in nSCR. In other words, stronger sympathetic activation during the speech phase was linked to alpha suppression and beta enhancement at the cortical level. However, it is important to note that correlation analyses do not allow causal inferences. The observed associations therefore reflect statistical relationships between neural and autonomic responses rather than direct physiological coupling.

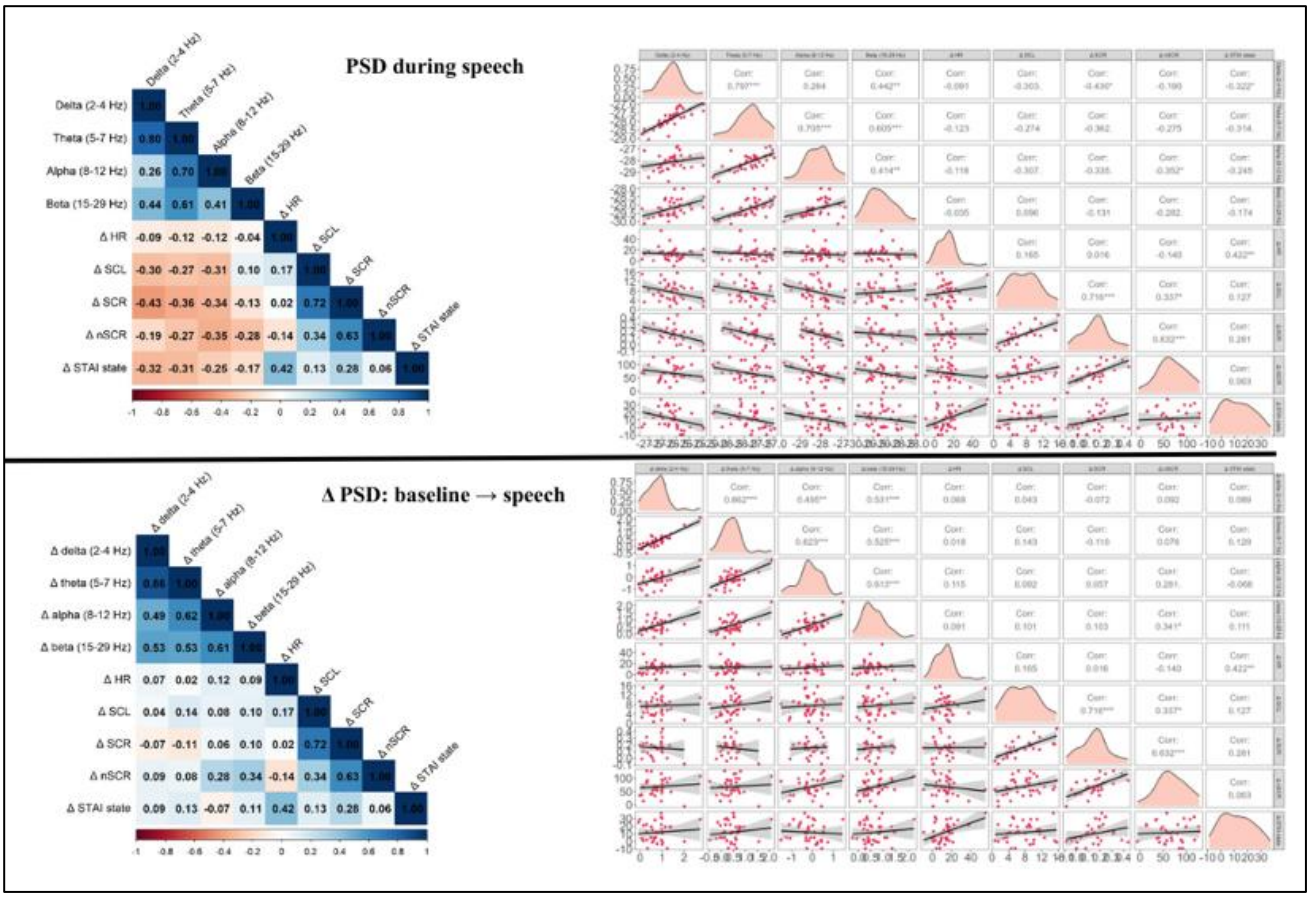


Figure 10. *Associations between EEG activity and stress-related Δ scores.* The lower panel displays Pearson correlation matrices and scatterplots showing associations between changes in

EEG power (ΔPSD: speech − baseline) in the alpha and beta bands and changes in physiological and subjective measures (ΔHR, ΔSCL, ΔSCR, ΔnSCR, ΔSTAI-S). Exploratory correlations for delta and theta bands are also displayed. The upper panel shows correlations between absolute EEG power during the speech phase and corresponding Δ scores. Color-coded heatmaps represent Pearson correlation coefficients, and scatterplots include fitted regression lines.

4.3 Discussion & Conclusion

4.3.1 Central & peripheral correlates of the stress response

The main goal of this thesis was to characterize how acute psychosocial stress modulates both oscillatory patterns in the central nervous system (EEG spectral power) and the autonomic system (ECG and EDA), using the TSST design. Overall, the findings suggest that the TSST elicited a pattern consistent with a stress response across central and peripheral measures, with clear modulation across experimental phases and a topographically distributed PSD across scalp electrode clusters.

On the peripheral side, the descriptive manipulation checks, as shown in the boxplots in Figure 3, indicated higher autonomic and subjective activation during the stress phases compared to baseline. This pattern is consistent with the TSST literature, which shows that social-evaluative threat reliably engages sympathetic arousal and subjective anxiety (Allen et al., 2017). Using both ECG and EDA allowed us to capture different components of the autonomic stress response. The increase in heart rate during the TSST likely reflects a shift in cardiac autonomic balance, characterized by reduced parasympathetic (vagal) activity together with increased sympathetic activation, a pattern commonly observed during psychosocial stress and performance situations (Allen et al., 2017; Berntson et al., 1997). In parallel, increases in SCL and phasic SCR indices (amplitude and nSCR) indicate heightened sympathetic activation at the level of the sweat glands. EDA reflects increased sympathetic outflow to eccrine sweat glands, which directly alters skin conductance (Boucsein, 2012). Because EDA is driven almost exclusively by sympathetic pathways, it provides a relatively specific index of sympathetic arousal. The convergence of these measures strengthens the validity of the stress induction, demonstrating that participants not only reported feeling stressed but also showed corresponding physiological responses.

On the central side, EEG power in canonical frequency bands showed phase modulation across the task. This overall trajectory fits well with a general interpretation of stress as a shift toward heightened vigilance and engagement during anticipation and acute stress, followed by partial normalization during recovery (Schubert et al., 2009; Vanhollebeke et al., 2022).

In addition, at the cluster level, the observed frontal and occipital cluster effects are consistent with the systematic review and meta-analysis by Vanhollebeke et al. (2022), which reported that acute psychosocial stress during the TSST is frequently associated with modulation of oscillatory power over frontal and posterior scalp regions across canonical frequency bands. In line with this literature, the present findings indicate stress-related topographical differences in scalp-recorded spectral power. Importantly, these effects reflect variations at the level of electrode clusters and should not be interpreted as evidence of specific cortical source involvement. Although the present analyses revealed cluster effects at the scalp level, these findings should be interpreted with caution, given the methodological specificities of EEG spatial resolution.

We should indeed consider that the scalp topographies do not provide a direct representation of the underlying cortical generators. This limitation is primarily related to the inverse problem in EEG source analysis. While it is possible to calculate how a known cortical source would project to the scalp (the forward problem), determining the exact neural sources responsible for an observed scalp distribution is fundamentally ill-posed (Grech et al., 2008; Michel & Murray, 2012). In practical terms, multiple different source configurations can produce similar scalp patterns, and without additional anatomical or mathematical constraints, a unique solution cannot be identified. Therefore, although frontal, central, and occipital clusters provide useful functional approximations, they cannot be equated with specific cortical regions. For example, increased frontal beta power at the scalp does not necessarily imply exclusive activation of the prefrontal cortex, as deeper or distributed sources may contribute to the observed pattern.

Alpha-band activity showed a distinct pattern compared to the Beta bands. Alpha power was higher at baseline than during preparation and speech, then increased again during recovery. Alpha oscillations are widely interpreted as reflecting functional inhibition of cortical regions, whereby increased alpha power reduces neuronal excitability and limits information processing in task-irrelevant areas (Klimesch, 1999). The inhibition–timing framework suggests that alpha rhythms

regulate information flow by suppressing task-irrelevant neural populations, shaping attention and sensory processing (Klimesch, 1999). Recent evidence suggests that alpha activity does not represent a single attentional mechanism but rather reflects multiple processes involved in attentional control (Cruz et al., 2025). During the preparation and speech phases of the TSST, participants engage in sustained performance monitoring, social-evaluative processing, and goal-directed cognitive control. These activities increase attentional demands and cortical excitability, typically resulting in alpha suppression. In this context, reduced alpha power may indicate heightened vigilance and greater allocation of cognitive resources to the socially threatening environment. The rebound in alpha power during recovery suggests a partial return of inhibitory control and reduced external engagement, consistent with a shift to a more internally focused or resting-state mode after stress exposure.

In contrast, Beta-band power increased significantly during the speech phase relative to baseline and preparation. Beta oscillations have been consistently associated with sustained cognitive engagement, active maintenance of task sets, and motor readiness (Engel & Fries, 2010; Spitzer & Haegens, 2017; Ray & Cole, 1985). In socially evaluative contexts such as the TSST, the speech phase requires continuous goal-directed behavior, performance monitoring, and regulation of verbal output under perceived observation. Increased beta power during this phase may therefore reflect heightened action readiness and sustained top–down control in response to social threat (Kamiński et al., 2012). From a broader neurocognitive perspective, beta oscillations are thought to support the maintenance of the "status quo" in task-relevant neural networks, stabilizing ongoing cognitive and behavioral states (Engel & Fries, 2010). Under acute stress, this stabilization may serve to maintain coherent speech production and goal-directed performance despite elevated arousal. Thus, the observed increase in beta during speech likely reflects intensified cognitive engagement and regulatory effort in response to social-evaluative stress. Acute stress is known to alter attentional processing by directing cognitive resources toward internally generated threat-related thoughts, such as concerns about performance or social evaluation. In this context, increased beta activity has been interpreted as a neural mechanism supporting compensatory cognitive control, helping to redirect attentional resources toward the ongoing task despite the presence of stress-related distraction. Consistent with this interpretation, previous research has shown that increases in beta-band activity following psychosocial stress

induction may reflect endogenous top-down modulation processes aimed at maintaining task performance (Palacios-García et al., 2021). The preparation phase showed intermediate neural and autonomic activation between baseline and speech, supporting the idea that stress responses begin before the actual performance component. This finding is consistent with the phase-resolved framework outlined in Chapter 3 and confirms that anticipation represents an active stage of stress engagement rather than a passive waiting period.

During recovery, beta power decreased relative to the speech phase ($p < .001$), indicating reduced neural activation once the stressor ended. However, power in these bands remained higher than baseline levels. This suggests that participants had begun to recover, but their neural activity had not yet fully returned to pre-stress levels within the 5-minute recovery period. This pattern is consistent with research showing that stress responses often decline after the task ends but may require more time to fully return to baseline (W. K. Goodman et al., 2017; Kirschbaum et al., 1993). From a functional perspective, the reduction in beta power from speech to recovery may reflect disengagement from sustained cognitive and motor demands once the socially evaluative task ended. Their decrease during recovery is therefore consistent with reduced performance-related engagement following completion of the speech phase (Jensen & Mazaheri, 2010a; Klimesch, 2012).

One framework that may help interpret the observed associations between neural and autonomic responses is the Neurovisceral Integration Model (Thayer et al., 2012; Thayer & Lane, 2000). This model proposes that cognitive, emotional, and autonomic processes are supported by a distributed network that includes prefrontal cortical regions, limbic structures, and brainstem autonomic centers. Within this network, higher-order cortical areas are thought to contribute to the regulation of physiological states by influencing subcortical and autonomic control systems. From this perspective, oscillatory activity measured with EEG may reflect cortical processes involved in monitoring environmental demands and adjusting behavioral and physiological responses during stress. In situations involving social evaluation, such as the TSST, individuals must simultaneously manage cognitive demands, emotional appraisal, and physiological arousal. Neural oscillations in specific frequency bands may therefore index the level of cognitive engagement and regulatory effort required to cope with the stressor. The correlations observed in the present study between

these oscillatory patterns and sympathetic activation may therefore reflect the parallel recruitment of cognitive and autonomic processes during acute stress.

Importantly, these associations should not be interpreted as evidence of direct causal interactions between neural oscillations and peripheral physiology. Instead, both neural and autonomic responses may reflect the influence of shared regulatory mechanisms engaged during social-evaluative threat.

4.3.2 Individual differences in brain–body associations

Beyond overall phase effects, the aim of this thesis was to examine individual differences in stress reactivity by testing whether variability in EEG activity relates to variability in autonomic and subjective stress responses, which are described in detail in the second hypothesis. Although group-level analyses revealed consistent phase effects, substantial variability in the magnitude of neural and autonomic responses was observed across individuals. Previous research has often reported heterogeneous and sometimes inconsistent neural findings during acute psychosocial stress (Vanhollebeke et al., 2022). Moreover, dissociations between central and peripheral stress responses have been documented, with subjective and physiological markers not always showing strong correspondence (Campbell & Ehlert, 2012), and brain–autonomic relationships appearing complex and variable across studies (Thayer et al., 2012). In contrast to this mixed evidence, the present results indicate that during the speech phase of the TSST, specific neural oscillatory changes were meaningfully associated with sympathetic activation. According to our findings, several significant associations emerged between EEG power and sympathetic or subjective reactivity measures.

First, lower alpha power during speech was associated with a stronger increase in the ΔnSCR, consistent with the inhibitory-gating interpretation of alpha activity. This suggests that individuals with reduced cortical inhibitory control exhibit greater sympathetic reactivity. Second, increases in beta power from baseline to speech (Δbeta) were positively associated with increases in ΔnSCR, indicating that stronger cortical engagement during stress co-occurred with greater sympathetic activation. The observed associations between reduced Alpha power and increased sympathetic activation should not be interpreted as evidence that cortical desynchronization directly causes

increased electrodermal activity or sympathetic sudomotor activation. Rather, these neural and peripheral changes may reflect the influence of a common upstream mechanism activated during social-evaluative threat. Thus, the current interpretation is consistent with established neurophysiological models of stress but does not constitute mechanistic proof of directional effects.

Overall, partial support for HP2 was found, with significant correlations between EEG activity, particularly in the alpha and beta bands, and sympathetic reactivity. These results suggest that neural oscillations during acute stress are linked to autonomic activation and subjective anxiety, indicating that EEG dynamics during the speech phase may index individual stress engagement and responsiveness.

Previous studies have shown mixed EEG results during stress, which may be due to differences in methods, tasks, and analysis (Vanhollebeke et al., 2022). In addition, individual differences—such as personality, anxiety, and past stress experience—can affect how people respond to stress (Allen et al., 2014). In the present study, although clear effects were observed at the group level, the relationship between brain and body responses varied across participants. For example, EEG activity, particularly in the alpha and beta bands, was related to sympathetic responses during the speech phase, but this was not consistent across all measures.

These findings suggest that neural and autonomic responses to stress are not uniform across individuals. Although the present results do not fully explain inconsistencies in the literature, they highlight the importance of considering individual differences, as they can influence study outcomes. Future research with larger samples and models that explicitly account for individual variability may help clarify these brain–body dynamics.

4.4 Limitations

Several limitations should be acknowledged when interpreting the present findings. First, the study did not include a non-stress control group. While baseline measurements served as an internal reference condition, the absence of a control task limits the ability to disentangle stress-specific effects from general task engagement or time-related changes. Future research could incorporate

a matched non-evaluative speech or cognitive control condition to better isolate the unique contribution of social-evaluative stress. The recovery phase lasted five minutes and was administered immediately after the speech phase. While this duration is consistent with many TSST protocols, some studies suggest that autonomic and neural systems may require a longer time window to fully return to baseline levels (Dickerson & Kemeny, 2004). Therefore, the recovery phase in the present design may reflect early post-stress adaptation rather than complete physiological normalization. Extending the recovery period in future studies would allow for a more precise characterization of stress-resolution dynamics.

Another limitation concerns the characteristics of the sample. Although the sample was not composed exclusively of university students, the majority of participants were students. This limits the generalizability of the findings. A sample largely composed of students may not adequately represent the broader population in terms of age, life experience, or socio-demographic diversity. Therefore, the results should be interpreted with caution when extending them to more diverse populations, and although the number of participants was sufficient to detect the main patterns of stress responses, the relatively small sample may reduce statistical power and limit the reliability and generalizability of the findings.

The TSST was not explicitly described in the recruitment materials; however, participation required volunteering for a laboratory study involving physiological recordings and exposure to an evaluative task, which may have influenced who chose to take part. Questionnaire data nevertheless indicate that the sample did not include individuals with particularly high levels of trait anxiety or worry. Instead, participants were generally within normative ranges. Consequently, the study may have underrepresented individuals with extreme stress vulnerability. Reduced variability in dispositional anxiety and worry could have limited the magnitude of individual-difference effects, particularly in correlational analyses examining brain–body associations. This may partly explain why neural and autonomic correlations were modest despite clear phase-dependent stress effects at the group level. A further limitation involves the interpretation of brain–body interactions. The present analyses were based on simple correlations between EEG power and autonomic indices and therefore do not allow conclusions about dynamic coupling or directional influences between neural and peripheral systems. Although significant associations were observed, correlational findings do not capture the temporal coordination or causal interplay

underlying stress regulation. It remains unclear whether changes in cortical oscillatory activity precede autonomic activation, whether peripheral signals influence neural dynamics, or whether both reflect a shared regulatory mechanism.

A related limitation is the lack of hormonal measures, such as cortisol, which would allow assessment of the HPA axis activation. The present study focused on autonomic (ECG, EDA) and neural (EEG) indices of stress, but did not capture endocrine responses. Including salivary cortisol in future multimodal designs would provide a more comprehensive characterization of stress system coordination. Finally, speech performance during the TSST was not behaviorally quantified (e.g., fluency, pause duration, vocal stress markers). Including objective behavioral performance metrics would allow examination of how neural and autonomic responses relate to task execution quality, potentially clarifying whether stronger physiological activation reflects adaptive engagement or maladaptive dysregulation.

4.5 Future directions

Future research should include larger and more heterogeneous samples to improve the generalizability of the findings. Expanding recruitment beyond predominantly student populations would enable a broader, more representative sample. Including participants with a wider range of trait levels, particularly individuals within subclinical or clinical ranges, may help capture the full variability of the construct under investigation and better clarify its interaction with both physiological and psychological processes. Future studies should integrate hormonal measures, such as cortisol, to examine coordination among neural, autonomic, and endocrine stress systems. Longer recovery periods would clarify whether central and peripheral systems normalize in parallel or exhibit dissociated recovery trajectories. Additionally, incorporating behavioral performance measures and computational modeling approaches may further elucidate the regulatory mechanisms underlying individual differences in stress-related brain–body associations.

Although the present study was conducted in a healthy sample, the findings may have potential implications for anxiety disorders, where exaggerated stress reactivity and hyperarousal are central features. Previous research suggests that specific EEG patterns, such as increased frontal theta

activity or altered alpha dynamics, are associated with anxiety symptom severity and treatment response (Newson & Thiagarajan, 2019). In this context, the phase-specific oscillatory changes observed in the current study may represent candidate neural markers of stress sensitivity. Importantly, in anxiety disorders, similar patterns of heightened physiological reactivity are often observed. Therefore, it is possible that stress-related EEG profiles, such as those identified here, could eventually contribute to identifying individuals with exaggerated stress responsivity or predicting treatment response. For example, some studies suggest that quantitative EEG (qEEG) markers may help predict pharmacological response in anxiety disorders (Lee et al., 2023). While our study was not designed to test clinical prediction, the observed brain–autonomic associations suggest that multimodal markers could be explored as pretreatment indicators in future clinical research. Future research should therefore test whether the phase-specific EEG markers observed here generalize to individuals with anxiety disorders, and whether they can predict symptom severity, relapse risk, or treatment outcomes. Longitudinal designs and intervention studies would be particularly informative. Ultimately, while the present findings remain preliminary from a clinical perspective, they provide a foundation for exploring EEG-based biomarkers of stress reactivity in anxiety-related conditions.

Future research should move beyond simple correlations and examine whether stress-related EEG features can predict autonomic and subjective responses at the individual level. Multivariate and machine learning approaches may allow researchers to test whether patterns of neural activity anticipate changes in sympathetic arousal, or whether peripheral signals predict cortical modulation across stress phases. Such predictive modeling could clarify directional relationships between central and autonomic systems and may eventually contribute to identifying individuals with heightened stress vulnerability. However, these approaches require replication in larger samples and careful validation before clinical translation can be considered.

Another important methodological advancement involves moving beyond scalp-level spectral power toward source localization and network-based analyses. Although the present findings reflect topographical differences in electrode clusters, scalp-recorded EEG does not directly indicate the cortical generators of observed oscillatory changes. Future studies should therefore apply source reconstruction methods (e.g., inverse modeling techniques) to estimate the cortical regions contributing to stress-related alpha suppression and beta enhancement. This would help

clarify whether prefrontal, parietal, or limbic-related cortical networks are differentially engaged during anticipation, acute stress, and recovery. Additionally, functional connectivity metrics could reveal how communication between frontal and posterior regions evolves across stress phases. Such approaches would provide a more mechanistic understanding of how distributed neural networks coordinate during acute psychosocial stress. Overall, expanding multimodal and phase-resolved approaches across larger, more diverse samples will enhance understanding of how acute psychosocial stress unfolds across systems and individuals.

Future studies should also investigate additional EEG frequency bands, particularly theta and gamma, which were not extensively examined in the present analysis. These oscillations have been linked to processes such as cognitive control, emotional regulation, and large-scale neural communication. Exploring their modulation during different phases of the TSST, as well as their relationship with autonomic measures, may provide a more comprehensive understanding of the neural dynamics underlying acute stress responses.

An additional methodological development involves integrating immersive virtual reality (VR) paradigms into stress research. Virtual reality environments can enhance ecological validity by increasing perceived presence and social evaluation, thereby eliciting robust physiological and subjective stress responses comparable to those in traditional laboratory paradigms (Pan & Hamilton, 2018). At the same time, VR allows precise experimental control over environmental parameters, ensuring that all participants are exposed to standardized and reproducible stress conditions. This combination of immersion and experimental control may reduce variability related to experimenter differences while preserving the social-evaluative component central to psychosocial stress induction.

Bibliography

Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology*, *34*(10), 1423–1436. https://doi.org/10.1016/j.psyneuen.2009.06.011

Ahmed, M. H., Panchookian, J., Grillo, M., Weerasinghe, Y., Taebi, A., Qadri, F., Gamage, P., & Kaya, M. (2025). Stress Classification Through Simultaneous EEG, Heart Rate Variability, and EMG Monitoring. *2025 IEEE Medical Measurements & Applications (MeMeA)*, 1–6. https://doi.org/10.1109/MeMeA65319.2025.11068019

Allen, A. P., Kennedy, P. J., Cryan, J. F., Dinan, T. G., & Clarke, G. (2014). Biological and psychological markers of stress in humans: Focus on the Trier Social Stress Test. *Neuroscience & Biobehavioral Reviews*, *38*, 94–124. https://doi.org/10.1016/j.neubiorev.2013.11.005

Allen, A. P., Kennedy, P. J., Dockray, S., Cryan, J. F., Dinan, T. G., & Clarke, G. (2017). The Trier Social Stress Test: Principles and practice. *Neurobiology of Stress*, *6*, 113–126. https://doi.org/10.1016/j.ynstr.2016.11.001

Al-Shargie, F., Kiguchi, M., Badruddin, N., Dass, S. C., Hani, A. F. M., & Tang, T. B. (2016). Mental stress assessment using simultaneous measurement of EEG and fNIRS. *Biomedical Optics Express*, *7*(10), 3882. https://doi.org/10.1364/BOE.7.003882

Angioletti, L., Rovelli, K., & Balconi, M. (2025). Be ready to manage stress “Before” and “After” a critical event. What the EEG and autonomic correlates tell us. *Brain and Cognition*, *183*, 106244. https://doi.org/10.1016/j.bandc.2024.106244

Appelhans, B. M., & Luecken, L. J. (2006). Heart Rate Variability as an Index of Regulated Emotional Responding. *Review of General Psychology*, *10*(3), 229–240. https://doi.org/10.1037/1089-2680.10.3.229

Arnsten, A. F. T. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature Reviews Neuroscience*, *10*(6), 410–422. https://doi.org/10.1038/nrn2648

Arsalan, A., & Majid, M. (2021). Human stress classification during public speaking using physiological signals. *Computers in Biology and Medicine*, *133*, 104377. https://doi.org/10.1016/j.compbiomed.2021.104377

Barrett, L. F., & Simmons, W. K. (2015). Interoceptive predictions in the brain. *Nature Reviews Neuroscience*, *16*(7), 419–429. https://doi.org/10.1038/nrn3950

Barzegar, M., Jahromi, G. P., Meftahi, G. H., & Hatef, B. (2023). The Complexity of Electroencephalographic Signal Decreases during the Social Stress. *Journal of Medical Signals & Sensors*, *13*(1), 57–64. https://doi.org/10.4103/jmss.jmss_131_21

Bell, A. J., & Sejnowski, T. J. (1995). An Information-Maximization Approach to Blind Separation and Blind Deconvolution. *Neural Computation*, *7*(6), 1129–1159. https://doi.org/10.1162/neco.1995.7.6.1129

Benedek, M., & Kaernbach, C. (2010). A continuous measure of phasic electrodermal activity. *Journal of Neuroscience Methods*, *190*(1), 80–91. https://doi.org/10.1016/j.jneumeth.2010.04.028

Berntson, G. G., Thomas Bigger, J., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., Nagaraja, H. N., Porges, S. W., Saul, J. P., Stone, P. H., & Van Der Molen, M. W. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology*, *34*(6), 623–648. https://doi.org/10.1111/j.1469-8986.1997.tb02140.x

Berretz, G., Packheiser, J., Wolf, O. T., & Ocklenburg, S. (2022). Acute stress increases left hemispheric activity measured via changes in frontal alpha asymmetries. *iScience*, *25*(2), 103841. https://doi.org/10.1016/j.isci.2022.103841

Biondi, M., & Picardi, A. (1999). Psychological Stress and Neuroendocrine Function in Humans: The Last Two Decades of Research. *Psychotherapy and Psychosomatics*, *68*(3), 114–150. https://doi.org/10.1159/000012323

Blascovich, J., Mendes, W. B., Hunter, S. B., & Salomon, K. (1999). Social "facilitation" as challenge and threat. *Journal of Personality and Social Psychology*, *77*(1), 68–77. https://doi.org/10.1037/0022-3514.77.1.68

Bolger, N., & Schilling, E. A. (1991). Personality and the Problems of Everyday Life: The Role of Neuroticism In Exposure and Reactivity to Daily Stressors. *Journal of Personality*, *59*(3), 355–386. https://doi.org/10.1111/j.1467-6494.1991.tb00253.x

Boucsein, W. (2012). *Electrodermal Activity*. Springer US. https://doi.org/10.1007/978-1-4614-1126-0

Bradley, M. M., Miccoli, L., Escrig, M. A., & Lang, P. J. (2008). The pupil as a measure of emotional arousal and autonomic activation. *Psychophysiology*, *45*(4), 602–607. https://doi.org/10.1111/j.1469-8986.2008.00654.x

Brunyé, T. T., Goring, S. A., Navarro, E., Hart-Pomerantz, H., Grekin, S., McKinlay, A. M., & Plessow, F. (2025). Identifying the most effective acute stress induction methods for producing SAM- and HPA-related physiological responses: A meta-analysis. *Anxiety, Stress, & Coping*, *38*(3), 263–285. https://doi.org/10.1080/10615806.2025.2450620

Burke, H. M., Davis, M. C., Otte, C., & Mohr, D. C. (2005). Depression and cortisol responses to psychological stress: A meta-analysis. *Psychoneuroendocrinology*, *30*(9), 846–856. https://doi.org/10.1016/j.psyneuen.2005.02.010

Buske-Kirschbaum, A., Jobst, S., Wustmans, A., Kirschbaum, C., Rauh, W., & Hellhammer, D. (1997). Attenuated Free Cortisol Response to Psychosocial Stress in Children with

Atopic Dermatitis: *Psychosomatic Medicine*, *59*(4), 419–426. https://doi.org/10.1097/00006842-199707000-00012

Cabrera, A., Kolacz, J., Pailhez, G., Bulbena-Cabre, A., Bulbena, A., & Porges, S. W. (2018). Assessing body awareness and autonomic reactivity: Factor structure and psychometric properties of the Body Perception Questionnaire-Short Form (BPQ-SF). *International Journal of Methods in Psychiatric Research*, *27*(2), e1596. https://doi.org/10.1002/mpr.1596

Campbell, J., & Ehlert, U. (2012). Acute psychosocial stress: Does the emotional stress response correspond with physiological responses? *Psychoneuroendocrinology*, *37*(8), 1111–1134. https://doi.org/10.1016/j.psyneuen.2011.12.010

Can, Y. S., Arnrich, B., & Ersoy, C. (2018). Evaluation of an integrated system of wearable physiological sensors for stress monitoring in working environments using biological markers. *IEEE Transactions on Biomedical Engineering, 65*(8), 1748–1758.

Carleton, R. N., Norton, M. A. P. J., & Asmundson, G. J. G. (2007). Fearing the unknown: A short version of the Intolerance of Uncertainty Scale. *Journal of Anxiety Disorders*, *21*(1), 105–117. https://doi.org/10.1016/j.janxdis.2006.03.014

Chalmers, J. A., Quintana, D. S., Abbott, M. J.-A., & Kemp, A. H. (2014). Anxiety Disorders are Associated with Reduced Heart Rate Variability: A Meta-Analysis. *Frontiers in Psychiatry*, *5*. https://doi.org/10.3389/fpsyt.2014.00080

Chida, Y., & Hamer, M. (2008). Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: A quantitative review of 30 years of investigations. *Psychological Bulletin*, *134*(6), 829–885. https://doi.org/10.1037/a0013342

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nature Reviews Endocrinology*, *5*(7), 374–381. https://doi.org/10.1038/nrendo.2009.106

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A Global Measure of Perceived Stress. *Journal of Health and Social Behavior*, *24*(4), 385. https://doi.org/10.2307/2136404

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nature Reviews Neuroscience*, *3*(8), 655–666. https://doi.org/10.1038/nrn894

Cruz, G., Melcón, M., Sutandi, L., Matias Palva, J., Palva, S., & Thut, G. (2025). Oscillatory Brain Activity in the Canonical Alpha-Band Conceals Distinct Mechanisms in Attention. *The Journal of Neuroscience*, *45*(1), e0918242024. https://doi.org/10.1523/JNEUROSCI.0918-24.2024

Dawson, M. E., Schell, A. M., Filion, D. L., & Berntson, G. G. (2007). The Electrodermal System. In J. T. Cacioppo, L. G. Tassinary, & G. Berntson (Eds.), *Handbook of Psychophysiology* (3rd ed., pp. 157–181). Cambridge University Press. https://doi.org/10.1017/CBO9780511546396.007

De Cheveigné, A. (2020). ZapLine: A simple and effective method to remove power line artifacts. *NeuroImage*, *207*, 116356. https://doi.org/10.1016/j.neuroimage.2019.116356

De Kloet, E. R., Joëls, M., & Holsboer, F. (2005a). Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*, *6*(6), 463–475. https://doi.org/10.1038/nrn1683

Dhabhar, F. S. (2014). Effects of stress on immune function: The good, the bad, and the beautiful. *Immunologic Research*, *58*(2–3), 193–210. https://doi.org/10.1007/s12026-014-8517-0

Dickerson, S. S., & Kemeny, M. E. (2004). Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research. *Psychological Bulletin*, *130*(3), 355–391. https://doi.org/10.1037/0033-2909.130.3.355

Ehrhardt, N. M., Fietz, J., Kopf-Beck, J., Kappelmann, N., & Brem, A. (2022). Separating EEG correlates of stress: Cognitive effort, time pressure, and social-evaluative threat. *European Journal of Neuroscience*, *55*(9–10), 2464–2473. https://doi.org/10.1111/ejn.15211

Engel, A. K., & Fries, P. (2010). Beta-band oscillations—Signalling the status quo? *Current Opinion in Neurobiology*, *20*(2), 156–165. https://doi.org/10.1016/j.conb.2010.02.015

Essau, C. A., Sasagawa, S., & Ollendick, T. H. (2010). The facets of anxiety sensitivity in adolescents. *Journal of Anxiety Disorders*, *24*(1), 23–29. https://doi.org/10.1016/j.janxdis.2009.08.001

Eysenck, M. W., Derakshan, N., Santos, R., & Calvo, M. G. (2007). Anxiety and cognitive performance: Attentional control theory. *Emotion*, *7*(2), 336–353. https://doi.org/10.1037/1528-3542.7.2.336

Fazli, S., Mehnert, J., Steinbrink, J., Curio, G., Villringer, A., Müller, K.-R., & Blankertz, B. (2012). Enhanced performance by a hybrid NIRS–EEG brain computer interface. *NeuroImage*, *59*(1), 519–529. https://doi.org/10.1016/j.neuroimage.2011.07.084

Ferree, T. C. (2006). Spherical Splines and Average Referencing in Scalp Electroencephalography. *Brain Topography*, *19*(1–2), 43–52. https://doi.org/10.1007/s10548-006-0011-0

Fiksdal, A., Hanlin, L., Kuras, Y., Gianferante, D., Chen, X., Thoma, M. V., & Rohleder, N. (2019). Associations between symptoms of depression and anxiety and cortisol responses

to and recovery from acute stress. *Psychoneuroendocrinology*, *102*, 44–52. https://doi.org/10.1016/j.psyneuen.2018.11.035

Folkman, S., & Lazarus, R. S. (1985). If it changes it must be a process: Study of emotion and coping during three stages of a college examination. *Journal of Personality and Social Psychology*, *48*(1), 150–170. https://doi.org/10.1037/0022-3514.48.1.150

Freeston, M. H., Rhéaume, J., Letarte, H., Dugas, M. J., & Ladouceur, R. (1994). Why do people worry? *Personality and Individual Differences*, *17*(6), 791–802. https://doi.org/10.1016/0191-8869(94)90048-5

Fresco, D. M., Mennin, D. S., Heimberg, R. G., & Turk, C. L. (2003). Using the Penn State Worry Questionnaire to identify individuals with generalized anxiety disorder: A receiver operating characteristic analysis. *Journal of Behavior Therapy and Experimental Psychiatry*, *34*(3–4), 283–291. https://doi.org/10.1016/j.jbtep.2003.09.001

Gaab, J., Rohleder, N., Nater, U. M., & Ehlert, U. (2005). Psychological determinants of the cortisol stress response: The role of anticipatory cognitive appraisal. *Psychoneuroendocrinology*, *30*(6), 599–610. https://doi.org/10.1016/j.psyneuen.2005.02.001

Gärtner, M., Rohde-Liebenau, L., Grimm, S., & Bajbouj, M. (2014). Working memory-related frontal theta activity is decreased under acute stress. *Psychoneuroendocrinology*, *43*, 105–113. https://doi.org/10.1016/j.psyneuen.2014.02.009

Goodman, A. M., Wheelock, M. D., Harnett, N. G., Davis, E. S., Mrug, S., Deshpande, G., & Knight, D. C. (2022). Stress-Induced Changes in Effective Connectivity During Regulation of the Emotional Response to Threat. *Brain Connectivity*, *12*(7), 629–638. https://doi.org/10.1089/brain.2021.0062

Goodman, W. K., Janson, J., & Wolf, J. M. (2017). Meta-analytical assessment of the effects of protocol variations on cortisol responses to the Trier Social Stress Test. *Psychoneuroendocrinology*, *80*, 26–35. https://doi.org/10.1016/j.psyneuen.2017.02.030

Grech, R., Cassar, T., Muscat, J., Camilleri, K. P., Fabri, S. G., Zervakis, M., Xanthopoulos, P., Sakkalis, V., & Vanrumste, B. (2008). Review on solving the inverse problem in EEG source analysis. *Journal of NeuroEngineering and Rehabilitation*, *5*(1), 25. https://doi.org/10.1186/1743-0003-5-25

Gross, J. J., & John, O. P. (2003). Individual differences in two emotion regulation processes: Implications for affect, relationships, and well-being. *Journal of Personality and Social Psychology*, *85*(2), 348–362. https://doi.org/10.1037/0022-3514.85.2.348

Gu, H., Lei, Y., Yao, Y., Chen, C., & Liu, C. (2025). Physiological and psychological responses to acute stress: A meta-analysis of the 171 studies of Trier Social Stress Test including 8452 healthy adults. *Psychoneuroendocrinology*, *180*, 107566. https://doi.org/10.1016/j.psyneuen.2025.107566

Gu, H., Ma, X., Zhao, J., & Liu, C. (2022). A meta-analysis of salivary cortisol responses in the Trier Social Stress Test to evaluate the effects of speech topics, sex, and sample size. *Comprehensive Psychoneuroendocrinology*, *10*, 100125. https://doi.org/10.1016/j.cpnec.2022.100125

Gunnar, M. R., Reid, B. M., Donzella, B., Miller, Z. R., Gardow, S., Tsakonas, N. C., Thomas, K. M., DeJoseph, M., & Bendezú, J. J. (2021). Validation of an online version of the Trier Social Stress Test in a study of adolescents. *Psychoneuroendocrinology*, *125*, 105111. https://doi.org/10.1016/j.psyneuen.2020.105111

Guo, L.-N., Zhao, R.-L., Ren, A.-H., Niu, L.-X., & Zhang, Y.-L. (2019). Stress Recovery of Campus Street Trees as Visual Stimuli on Graduate Students in Autumn. *International Journal of Environmental Research and Public Health*, *17*(1), 148. https://doi.org/10.3390/ijerph17010148

Hafeez, M. A., Shakil, S., & Jangsher, S. (2018). Stress Effects on Exam Performance using EEG. *2018 14th International Conference on Emerging Technologies (ICET)*, 1–4. https://doi.org/10.1109/ICET.2018.8603652

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., & Myers, B. (2016). Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. In Y. S. Prakash (Ed.), *Comprehensive Physiology* (1st ed., pp. 603–621). Wiley. https://doi.org/10.1002/cphy.c150015

Hermans, E. J., Henckens, M. J. A. G., Joëls, M., & Fernández, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in Neurosciences*, *37*(6), 304–314. https://doi.org/10.1016/j.tins.2014.03.006

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., & Wolf, O. T. (2009). Neuroendocrine and psychometric evaluation of a placebo version of the 'Trier Social Stress Test.' *Psychoneuroendocrinology*, *34*(7), 1075–1086. https://doi.org/10.1016/j.psyneuen.2009.02.008

Holm, A., Lukander, K., Korpela, J., Sallinen, M., & Müller, K. M. I. (2009). Estimating Brain Load from the EEG. *The Scientific World JOURNAL*, *9*, 639–651. https://doi.org/10.1100/tsw.2009.83

Izhar, L. I., Roslan, N. S., Feng, Y. X., Faye, I., Ho, E. T. W., & Abdul Rahman, M. (2019). Classification of neuroticism using psychophysiological signals during speaking task

based on two different baseline measurements. *International Journal of Integrated Engineering, 11*(3).

Jensen, O., & Mazaheri, A. (2010a). Shaping Functional Architecture by Oscillatory Alpha Activity: Gating by Inhibition. *Frontiers in Human Neuroscience*, *4*. https://doi.org/10.3389/fnhum.2010.00186

Johnson, E. O., Kamilaris, T. C., Chrousos, G. P., & Gold, P. W. (1992). Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. *Neuroscience & Biobehavioral Reviews*, *16*(2), 115–130. https://doi.org/10.1016/S0149-7634(05)80175-7

Julian, L. J. (2011). Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). *Arthritis Care & Research*, *63*(S11). https://doi.org/10.1002/acr.20561

Juster, R.-P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience & Biobehavioral Reviews*, *35*(1), 2–16. https://doi.org/10.1016/j.neubiorev.2009.10.002

Kaldewaij, R., Koch, S. B. J., Volman, I., Toni, I., & Roelofs, K. (2016). On the Control of Social Approach–Avoidance Behavior: Neural and Endocrine Mechanisms. In M. Wöhr & S. Krach (Eds.), *Social Behavior from Rodents to Humans* (Vol. 30, pp. 275–293). Springer International Publishing. https://doi.org/10.1007/7854_2016_446

Kamiński, J., Brzezicka, A., Gola, M., & Wróbel, A. (2012). Beta band oscillations engagement in human alertness process. *International Journal of Psychophysiology*, *85*(1), 125–128. https://doi.org/10.1016/j.ijpsycho.2011.11.006

Katmah, R., Al-Shargie, F., Tariq, U., Babiloni, F., Al-Mughairbi, F., & Al-Nashash, H. (2021). A Review on Mental Stress Assessment Methods Using EEG Signals. *Sensors*, *21*(15), 5043. https://doi.org/10.3390/s21155043

Khalsa, S. S., Adolphs, R., Cameron, O. G., Critchley, H. D., Davenport, P. W., Feinstein, J. S., Feusner, J. D., Garfinkel, S. N., Lane, R. D., Mehling, W. E., Meuret, A. E., Nemeroff, C. B., Oppenheimer, S., Petzschner, F. H., Pollatos, O., Rhudy, J. L., Schramm, L. P., Simmons, W. K., Stein, M. B., … Zucker, N. (2018). Interoception and Mental Health: A Roadmap. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *3*(6), 501–513. https://doi.org/10.1016/j.bpsc.2017.12.004

Kiecolt-Glaser, J. K., Renna, M. E., Shrout, M. R., & Madison, A. A. (2020). Stress Reactivity: What Pushes Us Higher, Faster, and Longer—and Why It Matters. *Current Directions in Psychological Science*, *29*(5), 492–498. https://doi.org/10.1177/0963721420949521

Kim, H.-G., Cheon, E.-J., Bai, D.-S., Lee, Y. H., & Koo, B.-H. (2018). Stress and Heart Rate Variability: A Meta-Analysis and Review of the Literature. *Psychiatry Investigation*, *15*(3), 235–245. https://doi.org/10.30773/pi.2017.08.17

Kirschbaum, C., Pirke, K.-M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test' – A Tool for Investigating Psychobiological Stress Responses in a Laboratory Setting. *Neuropsychobiology*, *28*(1–2), 76–81. https://doi.org/10.1159/000119004

Klimesch, W. (1999). EEG alpha and theta oscillations reflect cognitive and memory performance: A review and analysis. *Brain Research Reviews*, *29*(2–3), 169–195. https://doi.org/10.1016/S0165-0173(98)00056-3

Klimesch, W. (2012). Alpha-band oscillations, attention, and controlled access to stored information. *Trends in Cognitive Sciences*, *16*(12), 606–617. https://doi.org/10.1016/j.tics.2012.10.007

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: A review. *Biological Psychology*, *84*(3), 394–421. https://doi.org/10.1016/j.biopsycho.2010.03.010

Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004). HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: Impact of age and gender. *Psychoneuroendocrinology*, *29*(1), 83–98. https://doi.org/10.1016/S0306-4530(02)00146-4

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). **lmerTest** Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(13). https://doi.org/10.18637/jss.v082.i13

Kyrou, I., & Tsigos, C. (2009). Stress hormones: Physiological stress and regulation of metabolism. *Current Opinion in Pharmacology*, *9*(6), 787–793. https://doi.org/10.1016/j.coph.2009.08.007

Laborde, S., Mosley, E., & Thayer, J. F. (2017). Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research – Recommendations for Experiment Planning, Data Analysis, and Data Reporting. *Frontiers in Psychology*, *08*. https://doi.org/10.3389/fpsyg.2017.00213

Larsen, J. T., Norris, C. J., & Cacioppo, J. T. (2003). Effects of positive and negative affect on electromyographic activity over *zygomaticus major* and *corrugator supercilii*. *Psychophysiology*, *40*(5), 776–785. https://doi.org/10.1111/1469-8986.00078

Lazarus, R. S. (1966). *Psychological stress and the coping process.* McGraw-Hill.

LeDoux, J. E. (2000a). Emotion Circuits in the Brain. *Annual Review of Neuroscience*, *23*(1), 155–184. https://doi.org/10.1146/annurev.neuro.23.1.155

Lenth, R. V. (2020). *Estimated marginal means, aka least-squares means*. R package version [1.8.9]. https://cran.r-project.org/package=emmeans

Liang, L., Chen, C. X., Chan, N. Y., Yau, S.-Y., Liu, Y., Li, S. X., Wing, Y. K., Lee, T. M.-C., & Hou, W. K. (2026). Predicting acute stress recovery: A resilience index of physiological responses to Trier Social Stress Test. *International Journal of Clinical and Health Psychology*, *26*(1), 100652. https://doi.org/10.1016/j.ijchp.2025.100652

Logothetis, N. K. (2008). What we can do and what we cannot do with fMRI. *Nature*, *453*(7197), 869–878. https://doi.org/10.1038/nature06976

Lohaus, A., Rueth, J.-E., Kater, M.-J., Werner, A., Lemola, S., & Schlarb, A. (2025). The Trier Social Stress Test in Adolescents: Associations With Stress Scale Data and Ecological Momentary Assessment. *European Journal of Health Psychology*, *32*(4), 187–198. https://doi.org/10.1027/2512-8442/a000183

Lovibond, P. F., & Lovibond, S. H. (1995). The structure of negative emotional states: Comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behaviour Research and Therapy*, *33*(3), 335–343. https://doi.org/10.1016/0005-7967(94)00075-U

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Mathewson, K. E., Lleras, A., Beck, D. M., Fabiani, M., Ro, T., & Gratton, G. (2011). Pulsed Out of Awareness: EEG Alpha Oscillations Represent a Pulsed-Inhibition of Ongoing

Cortical Processing. *Frontiers in Psychology*, *2*. https://doi.org/10.3389/fpsyg.2011.00099

Mayer, E. A. (2011). Gut feelings: The emerging biology of gut–brain communication. *Nature Reviews Neuroscience*, *12*(8), 453–466. https://doi.org/10.1038/nrn3071

Mcewen, B. S. (1998). Stress, Adaptation, and Disease: Allostasis and Allostatic Load. *Annals of the New York Academy of Sciences*, *840*(1), 33–44. https://doi.org/10.1111/j.1749-6632.1998.tb09546.x

McEwen, B. S. (2007). Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. *Physiological Reviews*, *87*(3), 873–904. https://doi.org/10.1152/physrev.00041.2006

McEwen, B. S., & Akil, H. (2020). Revisiting the Stress Concept: Implications for Affective Disorders. *The Journal of Neuroscience*, *40*(1), 12–21. https://doi.org/10.1523/JNEUROSCI.0733-19.2019

McEwen, B. S., & Morrison, J. H. (2013). The Brain on Stress: Vulnerability and Plasticity of the Prefrontal Cortex over the Life Course. *Neuron*, *79*(1), 16–29. https://doi.org/10.1016/j.neuron.2013.06.028

Medic, G., Wille, M., & Hemels, M. (2017). Short- and long-term health consequences of sleep disruption. *Nature and Science of Sleep*, *Volume 9*, 151–161. https://doi.org/10.2147/NSS.S134864

Meerlo, P., Sgoifo, A., & Suchecki, D. (2008). Restricted and disrupted sleep: Effects on autonomic function, neuroendocrine stress systems and stress responsivity. *Sleep Medicine Reviews*, *12*(3), 197–210. https://doi.org/10.1016/j.smrv.2007.07.007

Meyer, T. J., Miller, M. L., Metzger, R. L., & Borkovec, T. D. (1990). Development and validation of the penn state worry questionnaire. *Behaviour Research and Therapy*, *28*(6), 487–495. https://doi.org/10.1016/0005-7967(90)90135-6

Michel, C. M., & Murray, M. M. (2012). Towards the utilization of EEG as a brain imaging tool. *NeuroImage*, *61*(2), 371–385. https://doi.org/10.1016/j.neuroimage.2011.12.039

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin*, *133*(1), 25–45. https://doi.org/10.1037/0033-2909.133.1.25

Minguillon, J., Lopez-Gordo, M. A., & Pelayo, F. (2016). Stress Assessment by Prefrontal Relative Gamma. *Frontiers in Computational Neuroscience*, *10*. https://doi.org/10.3389/fncom.2016.00101

Minguillon, J., Lopez-Gordo, M. A., Renedo-Criado, D. A., Sanchez-Carrion, M. J., & Pelayo, F. (2017). Blue lighting accelerates post-stress relaxation: Results of a preliminary study. *PLOS ONE*, *12*(10), e0186399. https://doi.org/10.1371/journal.pone.0186399

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., & Cryan, J. F. (2014). The microbiome: Stress, health and disease. *Mammalian Genome*, *25*(1–2), 49–74. https://doi.org/10.1007/s00335-013-9488-5

Narvaez Linares, N. F., Charron, V., Ouimet, A. J., Labelle, P. R., & Plamondon, H. (2020). A systematic review of the Trier Social Stress Test methodology: Issues in promoting study comparison and replicable research. *Neurobiology of Stress*, *13*, 100235. https://doi.org/10.1016/j.ynstr.2020.100235

Palacios-García, I., Silva, J., Villena-González, M., Campos-Arteaga, G., Artigas-Vergara, C., Luarte, N., Rodríguez, E., & Bosman, C. A. (2021). Increase in Beta Power Reflects

Attentional Top-Down Modulation After Psychosocial Stress Induction. *Frontiers in Human Neuroscience*, *15*, 630813. https://doi.org/10.3389/fnhum.2021.630813

Pan, X., & Hamilton, A. F. D. C. (2018). Why and how to use virtual reality to study human social interaction: The challenges of exploring a new research landscape. *British Journal of Psychology*, *109*(3), 395–417. https://doi.org/10.1111/bjop.12290

Perrin, F., Pernier, J., Bertrand, O., & Echallier, J. F. (1989). Spherical splines for scalp potential and current density mapping. *Electroencephalography and Clinical Neurophysiology*, *72*(2), 184–187. https://doi.org/10.1016/0013-4694(89)90180-6

Perrin, S. L., Jay, S. M., Vincent, G. E., Sprajcer, M., Lack, L., Ferguson, S. A., & Vakulin, A. (2019). Waking qEEG to assess psychophysiological stress and alertness during simulated on-call conditions. *International Journal of Psychophysiology*, *141*, 93–100. https://doi.org/10.1016/j.ijpsycho.2019.04.001

Pessoa, L. (2009). How do emotion and motivation direct executive control? *Trends in Cognitive Sciences*, *13*(4), 160–166. https://doi.org/10.1016/j.tics.2009.01.006

Pfefferbaum, B., & North, C. S. (2020). Mental Health and the Covid-19 Pandemic. *New England Journal of Medicine*, *383*(6), 510–512. https://doi.org/10.1056/NEJMp2008017

Phelps, M. E. (2004). *PET: Physics, instrumentation, and scanners*. Springer. https://doi.org/10.1007/978-0-387-22529-6

Raidl, P., Wessner, B., Methlagl, M., & Csapo, R. (2025). Cross-Sectional Investigation of Acute Stress Responses to Two Different Laboratory Stress Tests in Male and Female Athletes. *Physiologia*, *6*(1), 2. https://doi.org/10.3390/physiologia6010002

Riaz, M., Guerra, P., & Gravina, R. (2025). EEG Sensor-Based Computational Model for Personality and Neurocognitive Health Analysis Under Social Stress. *Sensors*, *25*(24), 7634. https://doi.org/10.3390/s25247634

Ringgold, V., Burkhardt, F., Abel, L., Kurz, M., Müller, V., Richer, R., Eskofier, B. M., Shields, G. S., & Rohleder, N. (2025). Multimodal stress assessment: Connecting task-related changes in self-reported stress, salivary biomarkers, heart rate, and facial expressions in the context of the stress response to the Trier Social Stress Test. *Psychoneuroendocrinology*, *180*, 107560. https://doi.org/10.1016/j.psyneuen.2025.107560

Rohleder, N. (2019). Stress and inflammation – The need to address the gap in the transition between acute and chronic stress effects. *Psychoneuroendocrinology*, *105*, 164–171. https://doi.org/10.1016/j.psyneuen.2019.02.021

Salankar, N., & Qaisar, S. M. (2023). EEG based stress classification by using difference plots of variational modes and machine learning. *Journal of Ambient Intelligence and Humanized Computing*, *14*(12), 16347–16360. https://doi.org/10.1007/s12652-022-03856-3

Sandi, C., & Haller, J. (2015). Stress and the social brain: Behavioural effects and neurobiological mechanisms. *Nature Reviews Neuroscience*, *16*(5), 290–304. https://doi.org/10.1038/nrn3918

Schandry, R. (1981). Heart Beat Perception and Emotional Experience. *Psychophysiology*, *18*(4), 483–488. https://doi.org/10.1111/j.1469-8986.1981.tb02486.x

Schlotz, W., Yim, I. S., Zoccola, P. M., Jansen, L., & Schulz, P. (2011). The perceived stress reactivity scale: Measurement invariance, stability, and validity in three countries. *Psychological Assessment*, *23*(1), 80–94. https://doi.org/10.1037/a0021148

Schomer, D. L., Niedermeyer, E., & Lopes da Silva, F. H. (Eds.). (2011). *Niedermeyer's electroencephalography: Basic principles, clinical applications, and related fields* (6. ed). Wolters Kluwer, Lippincott Williams & Wilkins.

Schubert, C., Lambertz, M., Nelesen, R. A., Bardwell, W., Choi, J.-B., & Dimsdale, J. E. (2009). Effects of stress on heart rate complexity—A comparison between short-term and chronic stress. *Biological Psychology*, *80*(3), 325–332. https://doi.org/10.1016/j.biopsycho.2008.11.005

Schüle, C., Baghai, T. C., Di Michele, F., Eser, D., Pasini, A., Schwarz, M., Rupprecht, R., & Romeo, E. (2007). Effects of combination treatment with mood stabilizers and mirtazapine on plasma concentrations of neuroactive steroids in depressed patients. *Psychoneuroendocrinology*, *32*(6), 669–680. https://doi.org/10.1016/j.psyneuen.2007.04.004

Schwabe, L., Haddad, L., & Schachinger, H. (2008). HPA axis activation by a socially evaluated cold-pressor test. *Psychoneuroendocrinology*, *33*(6), 890–895. https://doi.org/10.1016/j.psyneuen.2008.03.001

Schwabe, L., & Wolf, O. T. (2013). Stress and multiple memory systems: From 'thinking' to 'doing.' *Trends in Cognitive Sciences*, *17*(2), 60–68. https://doi.org/10.1016/j.tics.2012.12.001

Seeman, T. E., Singer, B., Wilkinson, C. W., & Bruce McEwen. (2001). Gender differences in age-related changes in HPA axis reactivity. *Psychoneuroendocrinology*, *26*(3), 225–240. https://doi.org/10.1016/S0306-4530(00)00043-3

Seery, M. D. (2011). Resilience: A Silver Lining to Experiencing Adverse Life Events? *Current Directions in Psychological Science*, *20*(6), 390–394. https://doi.org/10.1177/0963721411424740

Selye, H. (1965). The stress syndrome. *AJN, American Journal of Nursing, 65*(3), 97–99.

Seth, A. K. (2013). Interoceptive inference, emotion, and the embodied self. *Trends in Cognitive Sciences*, *17*(11), 565–573. https://doi.org/10.1016/j.tics.2013.09.007

Shaffer, F., & Ginsberg, J. P. (2017). An Overview of Heart Rate Variability Metrics and Norms. *Frontiers in Public Health*, *5*, 258. https://doi.org/10.3389/fpubh.2017.00258

Shields, G. S., Sazma, M. A., & Yonelinas, A. P. (2016). The effects of acute stress on core executive functions: A meta-analysis and comparison with cortisol. *Neuroscience & Biobehavioral Reviews*, *68*, 651–668. https://doi.org/10.1016/j.neubiorev.2016.06.038

Smeets, T., Cornelisse, S., Quaedflieg, C. W. E. M., Meyer, T., Jelicic, M., & Merckelbach, H. (2012). Introducing the Maastricht Acute Stress Test (MAST): A quick and non-invasive approach to elicit robust autonomic and glucocorticoid stress responses. *Psychoneuroendocrinology*, *37*(12), 1998–2008. https://doi.org/10.1016/j.psyneuen.2012.04.012

Smith, E. E., Reznik, S. J., Stewart, J. L., & Allen, J. J. B. (2017). Assessing and conceptualizing frontal EEG asymmetry: An updated primer on recording, processing, analyzing, and interpreting frontal alpha asymmetry. *International Journal of Psychophysiology*, *111*, 98–114. https://doi.org/10.1016/j.ijpsycho.2016.11.005

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). *Manual for the State–Trait Anxiety Inventory (Form Y1–Y2)*. Consulting Psychologists Press.

Spitzer, B., & Haegens, S. (2017). Beyond the Status Quo: A Role for Beta Oscillations in Endogenous Content (Re)Activation. *Eneuro*, *4*(4), ENEURO.0170-17.2017. https://doi.org/10.1523/ENEURO.0170-17.2017

Steptoe, A., Hamer, M., & Chida, Y. (2007). The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis. *Brain, Behavior, and Immunity*, *21*(7), 901–912. https://doi.org/10.1016/j.bbi.2007.03.011

Stone, J. V. (2002). Independent component analysis: An introduction. *Trends in Cognitive Sciences*, *6*(2), 59–64. https://doi.org/10.1016/S1364-6613(00)01813-1

Subhani, A. R., Likun Xia, Malik, A. S., & Othman, Z. (2013). Quantification of physiological disparities and task performance in stress and control conditions. *2013 35th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*, 2060–2063. https://doi.org/10.1109/EMBC.2013.6609937

Tarvainen, M. P., Niskanen, J.-P., Lipponen, J. A., Ranta-aho, P. O., & Karjalainen, P. A. (2014). Kubios HRV – Heart rate variability analysis software. *Computer Methods and Programs in Biomedicine*, *113*(1), 210–220. https://doi.org/10.1016/j.cmpb.2013.07.024

Taylor, S., Zvolensky, M. J., Cox, B. J., Deacon, B., Heimberg, R. G., Ledley, D. R., Abramowitz, J. S., Holaway, R. M., Sandin, B., Stewart, S. H., Coles, M., Eng, W., Daly, E. S., Arrindell, W. A., Bouvard, M., & Cardenas, S. J. (2007). Robust dimensions of anxiety sensitivity: Development and initial validation of the Anxiety Sensitivity Index-3. *Psychological Assessment*, *19*(2), 176–188. https://doi.org/10.1037/1040-3590.19.2.176

Thayer, J. F., Åhs, F., Fredrikson, M., Sollers, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a

marker of stress and health. *Neuroscience & Biobehavioral Reviews*, *36*(2), 747–756. https://doi.org/10.1016/j.neubiorev.2011.11.009

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders*, *61*(3), 201–216. https://doi.org/10.1016/S0165-0327(00)00338-4

Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart–brain connection: Further elaboration of a model of neurovisceral integration. *Neuroscience & Biobehavioral Reviews*, *33*(2), 81–88. https://doi.org/10.1016/j.neubiorev.2008.08.004

Tugade, M. M., & Fredrickson, B. L. (2004). Resilient Individuals Use Positive Emotions to Bounce Back From Negative Emotional Experiences. *Journal of Personality and Social Psychology*, *86*(2), 320–333. https://doi.org/10.1037/0022-3514.86.2.320

Turner, A. I., Smyth, N., Hall, S. J., Torres, S. J., Hussein, M., Jayasinghe, S. U., Ball, K., & Clow, A. J. (2020). Psychological stress reactivity and future health and disease outcomes: A systematic review of prospective evidence. *Psychoneuroendocrinology*, *114*, 104599. https://doi.org/10.1016/j.psyneuen.2020.104599

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience*, *10*(6), 397–409. https://doi.org/10.1038/nrn2647

Vanhollebeke, G., De Smet, S., De Raedt, R., Baeken, C., Van Mierlo, P., & Vanderhasselt, M.-A. (2022). The neural correlates of psychosocial stress: A systematic review and meta-analysis of spectral analysis EEG studies. *Neurobiology of Stress*, *18*, 100452. https://doi.org/10.1016/j.ynstr.2022.100452

Volume Contents and Author Index. (2006). *Psychoneuroendocrinology*, *31*(10), e1–e18. https://doi.org/10.1016/j.psyneuen.2006.11.001

Von Dawans, B., Kirschbaum, C., & Heinrichs, M. (2011). The Trier Social Stress Test for Groups (TSST-G): A new research tool for controlled simultaneous social stress exposure in a group format. *Psychoneuroendocrinology*, *36*(4), 514–522. https://doi.org/10.1016/j.psyneuen.2010.08.004

Welch, P. (1967). The use of fast Fourier transform for the estimation of power spectra: A method based on time averaging over short, modified periodograms. *IEEE Transactions on Audio and Electroacoustics*, *15*(2), 70–73. https://doi.org/10.1109/TAU.1967.1161901

Yan, B., Wang, Y., Yang, Y., Wu, D., Sun, K., & Xiao, W. (2024). EEG Evidence of Acute Stress Enhancing Inhibition Control by Increasing Attention. *Brain Sciences*, *14*(10), 1013. https://doi.org/10.3390/brainsci14101013

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T. P., & Sahebkar, A. (2017). The impact of stress on body function: A review. *EXCLI Journal; 16:Doc1057; ISSN 1611-2156*. https://doi.org/10.17179/EXCLI2017-480

Yerkes, R. M., & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology*, *18*(5), 459–482. https://doi.org/10.1002/cne.920180503

Zhou, J., Gennatas, E. D., Kramer, J. H., Miller, B. L., & Seeley, W. W. (2012). Predicting Regional Neurodegeneration from the Healthy Brain Functional Connectome. *Neuron*, *73*(6), 1216–1227. https://doi.org/10.1016/j.neuron.2012.03.004

Zimmer, P., Buttlar, B., Halbeisen, G., Walther, E., & Domes, G. (2019). Virtually stressed? A refined virtual reality adaptation of the Trier Social Stress Test (TSST) induces robust

endocrine responses. *Psychoneuroendocrinology*, *101*, 186–192. https://doi.org/10.1016/j.psyneuen.2018.11.010

Zintel, S., Schmidt, L. I., Neubauer, A. B., Stoffel, M., Rafiee, Y., Ditzen, B., & Sieverding, M. (2025). The role of sex and gender role self-concept in stress reactivity: Evidence from the Trier Social Stress Test (TSST). *Psychoneuroendocrinology*, *178*, 107480. https://doi.org/10.1016/j.psyneuen.2025.107480

Appendices

Appendix 1: EEG montage

The EEG montage was performed according to the following standardized procedure:

1. Head circumference was measured to select the appropriate net size.
2. The vertex location was determined as the midpoint between the inion–nasion and left–right preauricular (tragus) distances.
3. The EEG net was soaked in electrolyte solution for approximately 5 minutes prior to application.
4. The net was positioned on the participant's head by two researchers, ensuring correct alignment along the midline (Fz–Cz–Pz).
5. The net was connected to the amplifier, taking care to avoid damage to the pins.
6. Electrode impedances were checked, and additional electrolyte solution was applied using a pipette to electrodes with high impedance.
7. EEG signals were then recorded.
8. After recording, the net was disconnected from the amplifier with care.
9. The net was removed from the participant.
10. The net was rinsed gently under running water.
11. The net was immersed in disinfectant solution for a maximum of 10 minutes.
12. The net was rinsed again with running water.
13. Finally, the net was hung to air dry.

ECG and EDA montage
Preparation

Prior to electrode placement, the following preparation steps were completed:

1. The recording device was checked to ensure it was fully charged.
2. Materials for skin preparation and disinfection were prepared.
3. Cotton swabs, cotton pads, disinfectant, and abrasive paste were arranged for electrode application.
4. Five disposable electrodes were prepared (three for ECG and two for EDA).
5. Recording cables were prepared:

- ECG: three-electrode cable (blue connector)
- EDA: two-electrode cable (green connector)

EDA montage

The EDA montage was performed as follows:

1. Participants were instructed to wash their hands with soap, avoiding excessive friction.
2. Electrodes were placed on the middle phalanges of the non-dominant hand.
3. The cable was connected to the recording device (slot 2).
4. EDA signals were recorded.
5. After recording, electrodes were removed and disposed of appropriately.

Appendix 2: The Full STAI-S questioner

State Trait Anxiety Inventory

Read each statement and select the appropriate response to indicate how you feel right now, that is, at this very moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	1	2	3	4
	Not at all	**A little**	**Somewhat**	**Very Much So**
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I feel tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel uncomfortable	1	2	3	4
11. I feel self confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I feel jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4
16. I feel content	1	2	3	4
17. I am worried	1	2	3	4
18. I feel confused	1	2	3	4
19. I feel steady	1	2	3	4
20. I feel pleasant	1	2	3	4

Appendix 3: phase and cluster and their interaction effect on each TSST phase

EEG Band Power by Phase and Cluster

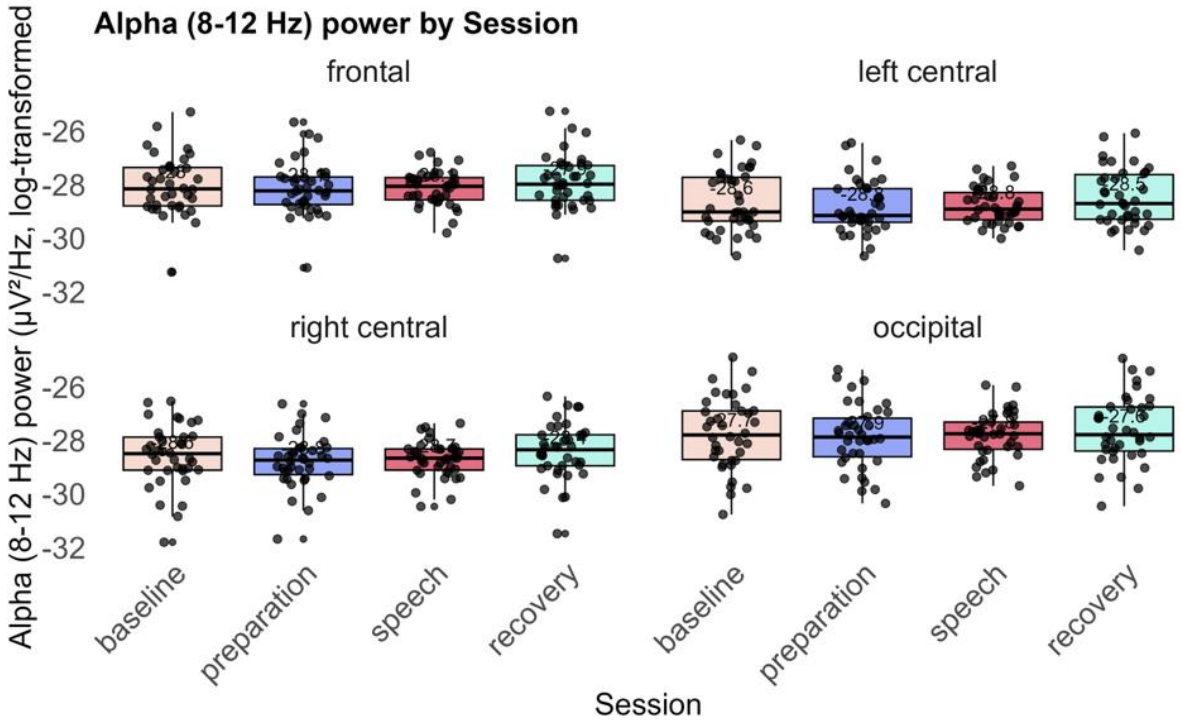
phase	cluster	delta_mean	delta_sd	theta_mean	theta_sd	alpha_mean	alpha_sd	beta_mean	beta_sd
base	front	-27.44689	0.6771010	-28.26137	0.7939402	-28.02144	1.0965661	-29.82725	0.7128689
base	centr_l	-28.27799	0.6113475	-28.97561	0.7116263	-28.61575	1.1177589	-30.42769	0.6825609
base	centr_r	-28.17120	0.6601400	-28.90434	0.7945613	-28.57088	1.1459008	-30.31727	0.7921157
base	occ	-27.42452	0.6289171	-28.30371	0.8012740	-27.74302	1.3543023	-29.77224	0.7158213
prep	front	-27.33034	0.5399894	-28.16402	0.6488688	-28.14625	0.9995300	-29.66505	0.5502346
prep	centr_l	-28.15368	0.4729973	-28.92588	0.5953173	-28.84885	0.9811724	-30.17912	0.5317163
prep	centr_r	-28.06047	0.5066752	-28.84198	0.6667866	-28.78937	0.9817423	-30.14414	0.6360571
prep	occ	-27.27865	0.5449534	-28.12714	0.6961012	-27.85520	1.2106856	-29.49800	0.6161969
spee	front	-26.66057	0.5053390	-27.83064	0.4979326	-28.13756	0.6561292	-29.20094	0.4999562
spee	centr_l	-27.42983	0.4394038	-28.59865	0.4701847	-28.78843	0.6731160	-29.52098	0.5952358
spee	centr_r	-27.41762	0.4143076	-28.53635	0.4682629	-28.70977	0.6849811	-29.54179	0.5915456
spee	occ	-26.61830	0.4620620	-27.73512	0.5247426	-27.81007	0.8724842	-28.89392	0.6016545
reco	front	-27.35230	0.5922522	-28.14081	0.7127280	-27.90428	1.0062964	-29.55426	0.5809555
reco	centr_l	-28.11264	0.5072785	-28.83730	0.6515292	-28.46535	1.0552937	-30.07688	0.5991750
reco	centr_r	-28.00542	0.5817594	-28.73851	0.7237346	-28.38865	1.0536231	-29.97730	0.7442700
reco	occ	-27.29224	0.6295879	-28.11749	0.7834581	-27.59572	1.2629083	-29.45477	0.6991626

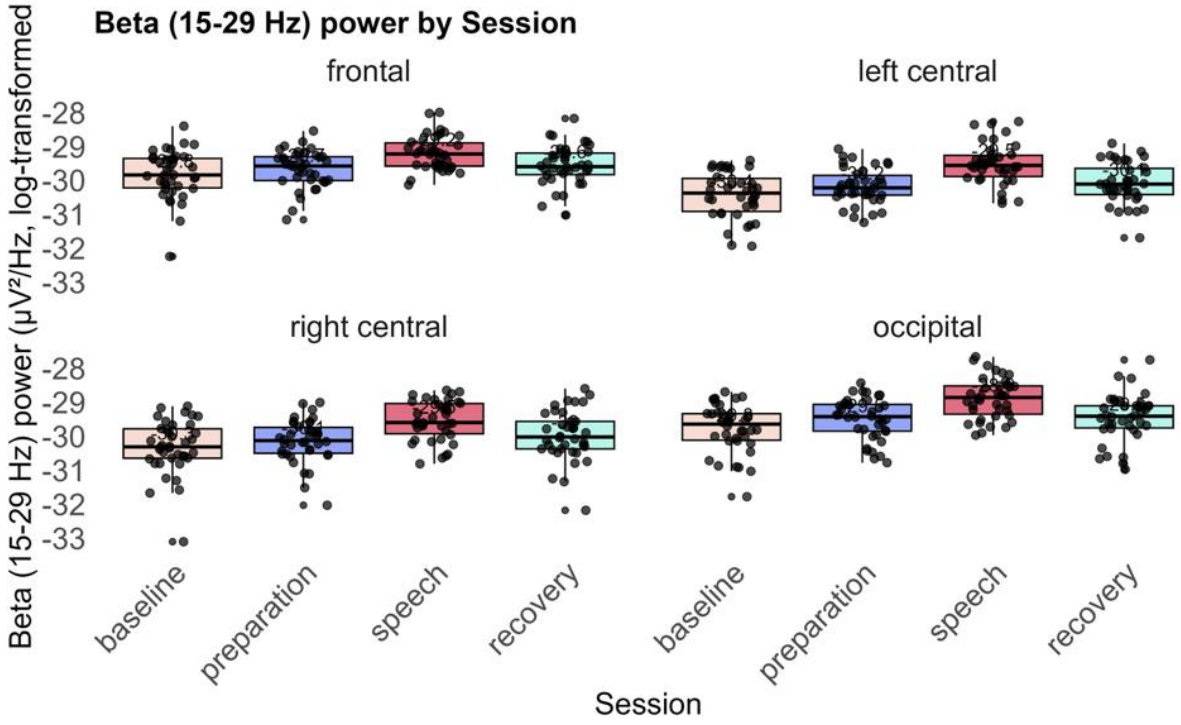
Appendix 4: Mean and SD of each EDA, ECG, and STAI for each Phase

Physiological & Psychological Measures by Phase

phase	meanHR_mean	meanHR_sd	meanSCL_mean	meanSCL_sd	meanSCR_mean	meanSCR_sd	nSCR_mean	nSCR_sd	STAI_state_mean	STAI_state_sd
base	84.65520	8.194924	6.868668	4.161861	0.1807700	0.1131193	33.44828	27.35606	40.44828	9.982894
prep	93.28763	10.963225	13.050268	4.851367	0.3239570	0.1553362	78.94444	26.47067	52.69444	11.055662
spee	96.00256	12.450907	13.174060	4.194551	0.3039326	0.1065382	92.80556	27.69143	51.02778	11.722875
reco	80.85043	9.542811	10.211950	4.286429	0.2067791	0.1130452	43.34286	30.37411	39.80000	10.234094

Appendix 5: Box plot of each cluster and phase for Alpha and Beta


Alpha (8-12 Hz) power by Session
frontal
left central
right central
occipital
Alpha (8-12 Hz) power (μV²/Hz, log-transformed
-26
-28
-30
-32
baseline
preparation
speech
recovery
Session


Beta (15-29 Hz) power by Session
frontal
left central
right central
occipital
Beta (15-29 Hz) power (μV²/Hz, log-transformed
-28
-29
-30
-31
-32
-33
baseline
preparation
speech
recovery
Session