# HEART RATE REACTIVITY IN STRESSED AND SLEEP DEPRIVED INDIVIDUALS

by

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THESIS

Presented to the Faculty of

The University of Houston-Clear Lake

In Partial Fulfillment

Of the Requirements

For the Degree

MASTER OF SCIENCE

in Psychology

THE UNIVERSITY OF HOUSTON-CLEAR LAKE

AUGUST, 2020

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## Dedication

For my parents and grandparents. War displaced them multiple times before they resettled in the U.S. The struggles they endured were all to make my American dreams come true. Thank you.

**Acknowledgements**

First and foremost, I want to express my sincerest appreciation to Dr. Georgina Moreno. My long-term goal has always been to become a researcher. However, you were the first professor that genuinely made me believe I was capable of becoming one. The guidance and support you gave me throughout my time in the Neuroscience and Behavior program has kept me motivated to hopefully further expand my academic career. It is safe to say I do not regret moving 957 miles just to attend school at UHCL. Thank you.

My appreciations also extend to my fellow colleagues in the Emotion and Decision-Making Lab, Michaella Petrosky, Ryan McAdams, Ellen Scott, and Allyssa Hashaw. Thank you for depriving yourselves of sleep just to assist me during this project. I definitely would not have been able to complete the experiments without all of you.

Other staff members at UHCL I would like to give thanks to is Ping-Hsun for helping us with the ELISA kits. Thank you to Dr. Nick Kelling for letting us use the lab space and making late-night runs whenever we needed your assistance in the lab. Finally, thank you to Dr. Angela Kelling for generously offering your time, patience, and expertise as a committee member throughout my thesis.

ABSTRACT

HEART RATE REACTIVITY IN STRESSED AND SLEEP DEPRIVED INDIVDIUALS

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Acute sleep deprivation and acute stress lead to similar changes in physiological and cognitive functioning. However, very little research has investigated the combined effects of acute sleep deprivation and acute stress on heart rate reactivity. The first aim of the current study was to compare delta heart rate in acutely sleep deprived and non-sleep deprived participants during an acute psychosocial stressor. It was hypothesized that sleep deprived participants would exhibit lower delta heart rate than well-rested participants. The second aim was to examine heart rate recovery after exposure to an acute stressor. It was hypothesized that sleep deprived individuals would experience delays in heart rate recovery immediately after the stressor. Lastly, an exploratory analysis was run to explore the relationship between cortisol and heart rate reactivity in sleep deprived and non-sleep deprived conditions. A sample of 15 healthy adults was included in the analyses. The participants either spent a night of total sleep deprivation ($N$=9) or a night of well-rested sleep ($N$=6). Beats-per-minute recordings were taken

before the acute stressor (pre-stress), during the acute stressor (stress), and immediately after the acute stressor (post-stress). Saliva samples were collected at the end of the pre-stress, stress, and post-stress time points. A significant difference was observed in estimated BPM variance between conditions, with sleep deprived individuals having less variability in their heart beats in comparison to non-sleep deprived individuals. There were no significant differences in delta heart rate or heart rate recovery between conditions. Moreover, exploratory analyses did not reveal a significant relationship between cortisol and estimated BPM. Taken together, the results of the present study suggest that acute sleep deprivation lowers estimated BPM variance. Future research should be conducted to better understand the extent to which acute psychosocial stress impacts physiological stress responses, namely heart rate reactivity and variance, in acutely sleep deprived individuals.

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# CHAPTER I:

# INTRODUCTION

## Context of the Problem

Sleep has a critical role in maintaining physical and psychological health in humans. For example, benefits of regular sleep include facilitative effects on memory consolidation for learning (Rasch & Born, 2013), maintenance of positive daytime mood and emotion (Gruber & Cassoff, 2014), and risk reduction of diseases such as hypertension and type II diabetes (Medic et al., 2017). On the contrary, sleep disturbances and restrictions can increase productions of inflammatory cytokines (Mullington et al., 2010), heighten risks of obesity (Medic et al., 2017), elevate cortisol reactivity (Horitsu et al., 2015), and increase susceptibility to cardiovascular diseases (Mullington et al., 2009).

Stress can often co-occur with sleep deprivation. During periods of stress, the HPA axis elicits cortisol as an adaptive response to stress (Ranabir & Reetu, 2011). Cortisol elevations due to acute stress have been associated with disruption of cognitive processes (Domes et al., 2004; McEwen, 2008). Acute stress mediates cardiovascular responses by accelerating heart rate (Clarke et al., 2014; Feldman, 1999) and increasing blood pressure (Grillon et al., 2007). These issues are often exacerbated by acute sleep deprivation (Tobaldini et al., 2013), where restricted sleep combined with stressful tasks prolongs the elevation of heart rate and blood pressure (Mezik et al., 2014). However, the combined effects of acute stress and acute sleep deprivation differ among individuals based on psychological variability (Van Dongen et al., 2005). It is essential to better understand how the combination of acute stress and acute sleep deprivation modulates both cardiovascular and hormonal homeostasis.

## Purpose of the Study

It has been established that both acute stress and acute sleep deprivation have negative effects on health. As previous research has suggested that the combined effects of sleep deprivation and stress potentially pose harm to the cardiovascular system, it is imperative to better understand heart rate and cortisol reactivity in acutely stressed and sleep deprived individuals. Aim 1 of the current study investigated the difference in delta heart rate between sleep deprived and non-sleep deprived participants during an acute psychosocial stressor. It was hypothesized that during the stressor, delta heat rate would decrease more in sleep deprived individuals than non-sleep deprived individuals. Aim 2 of the current study further investigated the impact of acute sleep deprivation on heart rate recovery after an acute stressor. It was hypothesized that sleep deprived participants would exhibit delayed heart rate recovery in comparison to non-sleep deprived participants. Lastly, Aim 3 explored the potential relationship between heart rate reactivity and cortisol reactivity in acutely sleep deprived and non-sleep deprived participants.

# CHAPTER II:

# LITERATURE REVIEW

## Heart Rate Reactivity

Stress is the body's response to threatening stimuli and affects an organism's biological equilibrium (Kranner et al., 2010). As a consequence, the body automatically attempts to preserve homeostasis by activating various biological defense mechanisms (Kranner et al., 2010; Perrson & Zakrisson, 2016). One of these protective systems includes the autonomic nervous system (ANS), an unconscious regulatory system which maintains allostasis primarily through the parasympathetic and sympathetic subsystems (Buijs & Van Eden, 2000; Hall et al., 2012). The sympathetic nervous system (SNS) responds to stressors by increasing heart rate, blood pressure, and sweat secretion (Esler, 2000; Gordan et al., 2015). To counter acute stress, the parasympathetic nervous system (PNS) inhibits stress responses produced by the SNS through lowering blood pressure and decelerating heart rate via vagus nerve innervation (Gordan et al., 2015).

The hypothalamic-pituitary-adrenal (HPA) axis, a neuroendocrine system which releases stress hormones (Jiang et al., 2019), has an indirect relationship with the ANS (Radley et al., 2011; Ulrich-Lai & Herman, 2009). Corticotropin-releasing factor (CRF) peptide hormones are hormones released from the hypothalamus that regulate activation of the HPA axis (Jiang et al., 2019; Smith & Vale, 2006) and mediate SNS activity (Rotenberg & McGrath, 2016; Zhang & Felder, 2008). CRFs have systemic modulations over the HPA axis and SNS (Yee et al., 2016), which indicates that ANS and HPA activity co-occur in response to stress. Consequently, understanding the fundamental principles of both stress response systems has generated two common techniques to evaluate the effects of stress on heart rate reactivity: heart rate variability (HRV) and heart rate recovery (Allen et al., 2014).

Heart rate reactivity is a broad term used to describe the involuntary change of heart rate induced by physical and mental stress (Cinciripini, 1986; Kudielka et al., 2004). Various procedures of capturing heart rate reactivity, such as myocardial oxygen (Heusch & Schulz, 2007), blood pressure (Franzen at al., 2011), and fluctuations in heart rate as measured by beats per minute (Zhong et al., 2005), are often utilized to evaluate stress levels. These results lead to the inference of underlying biological systems involved in stress modulation.

It has been established that heart rate increases during a stressor (De Geus & Van Doornen, 1993), especially a psychosocial stressor (Buske-Kirschbaum et al., 2002; Schubert et al., 2009). A well-validated acute psychosocial stressor commonly utilized in laboratory settings, the Trier Social Stress Task (TSST), has robust effects on heart rate acceleration (Allen et al., 2014; Mohammadi et al., 2019). Despite the abundance of research on the relationship between acute stress and heart rate reactivity, findings vary depending on the method used to measure heart rate reactivity. In addition to measuring average heart rate aceleration during acute stress, heart rate reactivity measurements such as HRV, heart rate recovery, and delta heart rate are also used to evaluate stress levels

## Stress and Heart Rate Reactivity

### Stress and Heart Rate Variability

HRV measures the change of time intervals between heart beats, or inter-beat-intervals, i.e., R-R intervals (HRV Task Force Report, 1996). HRV can be used to evaluate the impact of acute stress on the ANS as it assists in assessing parasympathetic and sympathetic activity (Kim et al., 2018; Schwarz et al., 2018). In HRV, frequency domain analyses are used to separate SNS and PNS activity based on the amount of input from each division (Rajendra et al., 2006). These domain frequencies enable researchers to momentarily assess ANS status during periods of acute stress (Kim et al., 2018;

Rajendra et al., 2006). High-frequency HRV reflects parasympathetic activation (HRV Task Force Report, 1996), and a greater high-frequency HRV after a stressor is associated with higher tolerance of stress (Kim et al., 2018). Acute psychosocial stress has been known to decrease HRV (Boesch et al., 2014), elevate low-frequency HRV (Castaldo et al., 2015), and reduce high-frequency HRV (Hjortskov et al., 2004). The rise of low-frequency HRV and decline of high-frequency HRV are indicative of dominant SNS activity and PNS withdrawal (HRV Task Force Report, 1996), which suggests that stress can cause an imbalance in the ANS. Moreover, research has shown that HRV can decrease during mental stress (Brugnera et al., 2018; Kim et al., 2018), as well as lower (Boesch et al., 2014) or remain unaffected during verbal psychosocial stress (Schwarz et al, 2018; Van Hedger et al., 2017).

**Stress and Heart Rate Recovery**

Heart rate recovery has traditionally been used to demonstrate the decline of heart rate after the first minute of exercise cessation (Ackland et al., 2019; Cole et al., 1999; Van De Vagte et al., 2018). A rapid reduction of heart rate is generally ideal during heart rate recovery as it is indicative of sufficient parasympathetic mediations stabilizing sympathetic activity (Cole et al., 1999; Kannankeril et al., 2004; Van De Vagte et al., 2018), and athletes have been found to have greater and accelerated recovery in comparison to non-athletes (Barak et al., 2011; Cole et al., 1999; Imai et al., 1994). Not only does inadequate recovery suggest lower exercise capacity (Cole et al., 1999), it is also connected to cardiovascular diseases, such as hypertension (Steptoe & Marmot, 2005) and all-cause mortality (Aneni et al., 2014; McCrory et al., 2016).

Exercise research has repeatedly reported vagus nerve innervation as the predominant mediator in heart rate recovery (Cole et al., 1999; Imai et al., 1994; Barbosa Lins et al., 2015). A speedy heart rate recovery suggests adequate parasympathetic

mitigation (Kannankeril et al., 2004), and robust heart rate recovery is commonly found in athletes, whereas blunted recovery has been associated with heart failure (Imai et al., 1994). However, blunted heart rate recovery can also be indicative of psychological stress (Verkuil, Brosschot, & Thayer, 2014). Accelerated heart rate and increased systolic blood pressure is primarily dependent on SNS activity (Gordan et al., 2015; Mancia & Grassi, 2014), and acute psychosocial stressors have been found to elicit both of these responses (Capisi et al., 2012; Carroll et al., 2012). Decrements of systolic blood pressure are positively associated with the gradation of heart rate recovery (Dogan et al., 2013), further corroborating that parasympathetic innervations are necessary for cardiovascular allostasis. Thus, blunted and delayed heart rate recovery are suggestive of insufficient sympathovagal imbalance; more specifically, they imply that there is vagal withdrawal and overstimulation of SNS activity (HRV Task Force Report, 1996).

Stress affects the cardiovascular system. Thus, it is necessary to examine heart rate recovery following acute psychosocial stress. Considerable effects seen in heart rate recovery may be dependent on the type of stressor used during experimentation. Social stressors, such as impromptu social simulations (Gordon et al., 2012), have been found to lead to heart rate acceleration; yet the research on heart rate recovery and acute stress is still inconsistent (Gordon et al., 2012; Loft et al., 2007; Verkuil et al., 2009). For example, slower heart rate recovery has been found to be induced by the TSST, (Verkuil et al., 2014), while other work has found that cognitive stressors may bring heart rate below pre-stress levels (Verkuil et al., 2009).

Other determinants of heart rate reactivity are biological factors such as age and gender (Uchino et al., 1999). Using age and gender as covariates, Kudielka and colleagues (2004) found that only younger women exposed to the TSST had elevated heart rate during both the stressor and recovery phase, while heart rate reactivity did not

differ among men and older women during and post-TSST. As for older adults, heart rate continuously increased during the recovery phase solely in women, but heart rate in older men returned back to baseline. Given the results of Kudielka and colleagues (2004), it is recommended that age and gender be considered during cardiovascular evaluations.

### Stress and Delta Heart Rate

Similar to the traditional use of heart rate recovery, delta heart rate has been commonly used in exercise health assessments, although abnormal delta values can also be characteristic of stress (De Weerth et al., 2007; Swaim, 2012). Delta heart rate is the calculated difference of heart rate after a change in body position, usually from a supine to orthostatic position (Swaim, 2012). In the context of acute stress, delta heart rate may be utilized to fine-tune average heart rate differences from a resting state to a stressed state. Although only a few studies focusing on acute psychosocial stress have used delta heart rate as a measurement, these experiments reported that delta values tend to increase during stressors involving public speech (De Weerth et al., 2007; Lennartsson et al., 2012). These findings also held true for psychosocial stress tasks such as the TSST and the Socially Evaluated Cold Pressor Task (Giles et al., 2014).

## Acute Sleep Deprivation

Sleep benefits an organism's physiological and psychological well-being by restoring a number of processes. The beneficial effects of sleep on biological mechanisms include metabolism maintenance (Punjabi & Polotsky, 1998) and immune defense (Besedovsky et al., 2012). Additionally, many cognitive functions are facilitated by sleep, such as memory consolidation (Poe et al., 2010; Scullin & Bliwise, 2015) and decision making (Bos et al., 2011). Multiple experiments have highlighted the potentially detrimental effects of acute sleep deprivation. The consequences of acute sleep deprivation include, but are not limited to, poorer cognitive performance (Killgore, 2010;

Rupp et al., 2012), decrements in positive mood regulation and maintenance (Killgore, 2010; Talbot et al., 2010) and reduction of alertness (Zhong et al., 2005). As the benefits of consistent and regular sleep are evident (Dworak et al., 2010; Kranner et al., 2010), acute sleep deprivation may have diminishing effects on health. Given that research has found that acute sleep deprivation increases risks of cardiovascular-related health issues by attenuating autonomic functions (Vicente et al., 2016; Zhong et al., 2005), it is important to understand how acute sleep deprivation impacts both the SNS and PNS.

**Acute Sleep Deprivation and the Cardiovascular System**

Similar to the effects of acute stress, research has suggested that acute sleep deprivation heightens SNS activity and reduces PNS activity (Mullington et al., 2009). Normal ranges of blood pressure are mediated by the baroreflex, a cardiovascular mechanism which manages cardiac contractility, vascular resistance, and heart rate (Duschek et al., 2013; Mullington et al., 2009). The baroreflex is mediated by both subdivisions of the ANS (Cowley, 1992). In a study by Ogawa et al. (2003), arterial baroreflex set point had increased in individuals who were sleep deprived for 24-hours, indicating that blood pressure had risen after a night of total sleep deprivation. Research has found that SNS activity decreases during sleep and PNS activity increases with sleep (Burgess et al., 1996; Fink et al., 2018). This corroborates with research that suggests SNS activity is predominantly influenced by sleep while the PNS is primarily influenced by circadian rhythm (Burgess, et al., 1997). Given that heart rate controls blood pressure, it indicates that heart rate reactivity is also activated through autonomic functions.

Although it is evident that SNS and PNS control heart rate reactivity, research on acute sleep deprivation and heart rate reactivity are variable. Some studies have indicated that heart rate is unaffected by a single day of total sleep deprivation (Dettoni et al., 2012; Kato et al., 2000). Other studies have found that a single night of total sleep deprivation

can induce elevations of systolic blood pressure (Franzen et al., 2011), increase heart rate while lying in a supine position, reduce average heart rate (Holmes et al., 2012), and reduce delta heart rate (Tolbadini et al., 2014). These cardiovascular characteristics are associated with excessive SNS and lower PNS activation (Castro-Diehl et al., 2016; Fisher & Paton, 2011). This suggests that wakefulness and sleep meditate ANS functions. However, despite the evidence that a single day of total sleep deprivation can potentially manifest harmful cardiovascular indicators, the relationship between acute sleep deprivation and heart rate reactivity (e.g., heart rate recovery, HRV, delta HR) is variable.

**Acute Sleep Deprivation and Heart Rate Recovery**

Given that heart rate recovery is a physiological biomarker, it has also been used to assess parasympathetic innervations during periods of sleep deprivation (Ackland et al., 2019; Okutucu et al., 2011). In a study conducted by Cincin et al. (2015), nurses and security officers participated in cardiovascular exercise under two conditions, after a night of adequate sleep and after working a night shift. The research concluded that in comparison to a night of regular sleep, participants had blunted heart rate recovery within the first thirty-seconds and first minute of the recovery phase when under acute sleep deprivation. However, this difference was not found in the last three minutes of exercise. Similarly, Imai and colleagues (1994) suggested that the first thirty-seconds of recovery is regulated through parasympathetic reactivation before receiving sympathetic support. Additionally, other research has shown that the strongest interaction between morality and heart rate recovery is within the first thirty-seconds of the recovery period (McCrory et al., 2016; Van De Vagte et al., 2018). Thus, it is imperative to monitor the initial thirty-seconds of recovery as it is indicative of vagal tone engagement (Cincin et al, 2015; Imai et al., 1994; McCrory et al., 2016).

## Acute Sleep Deprivation and Delta Heart Rate

There has been limited research on the relationship between acute sleep and delta heart rate. According to Tobaldini and colleagues (2014), acute sleep deprivation may disrupt autonomic balance and increase susceptibility to cardiovascular diseases. Being that delta heart rate is generally the difference in heart rate from a resting to standing position, orthostatic challenge tests can evaluate delta heart rate as it assesses cardiovascular adjustments from lying on one's back to actively standing up (Kirbiš et al., 2013). Research has shown that acute sleep deprivation elevates heart rate (Meerlo et al., 2008; Zhong et al., 2005), especially during orthostatic challenge (Robillard et al., 2011). However, Tobaldini and colleagues (2013) found that acute sleep deprivation blunted ANS activity during orthostatic challenge, which also reduced delta heart rate and delta HRV. This suggests that acute sleep deprivation may have opposing effects on both average and delta heart rate. Given this connection between delta heart rate and acute sleep deprivation, it is important to further explore how delta heart rate may be impacted by sleep deprivation, especially in the context of acute stress.

## Acute Sleep Deprivation, Stress, and Heart Rate Reactivity

More recently, research has started to further investigate the combined effects of acute sleep deprivation and stress on heart rate reactivity. Research has shown that when used as a cognitive stressor, the Stroop Color and Word Test increases heart rate and systolic blood pressure regardless of whether or not individuals were acutely sleep deprived (Franzen et al., 2011). Psychosocial acute stress has also been shown to delay heart rate recovery in sleep deprived individuals (Verkuil, Brosschot, & Thayer, 2014). To compare the effects of acute mental and physical stress under acute sleep deprivation, Yang and colleagues (2012) had acutely sleep deprived and non-sleep deprived participants subjected to two stressors one month apart, an acute mental stressor and an

acute physical stressor. It was found that participants in the sleep deprived condition had prolonged heightened heart rate recovery after both stressors, albeit recovery took longer to return to baseline after the mental stressor. To corroborate Yang and colleagues (2012), research on sleep deprivation has revealed that after exposure to an acute cognitive and social stressor, individuals who sleep less on average tend to have poorer heart rate recovery within the first two minutes of the recovery period (Mezick et al., 2014). Despite the evidence that chronic sleep deprivation is associated with delays in heart rate recovery after an acute stressor (Mezick et al., 2014), more research must be conducted to better understand how acute sleep deprivation mediates heart rate recovery after acute stress.

## Cortisol

Adrenocorticotropic hormones (ACTH) secreted from the anterior pituitary gland stimulates the zona fasciculata cells located in the adrenal gland, causing downstream synthesis of steroid hormones known as cortisol (Lightman et al., 2020). As ACTH generally peaks during the early mornings (Around 8:00 am), it remains relatively low throughout the day (Oster et al., 2017) and depletes to its lowest level two hours after the onset of sleep during nocturnal hours (Chan & Debono, 2010). The amount of cortisol released in the bloodstream is approximately proportional to ACTH production. (Balbo et al., 2010). Cortisol increases alertness and adaptation to threatening stimuli (Lightman et al., 2020). Due to its importance, diurnal rhythms of cortisol are necessary for regulating sleep-wake cycles and maintaining psychophysiological health.

As a primary stress response system (Smith & Vale, 2006), the HPA axis synthesizes cortisol to modulate anti-inflammatory, immune, and cardiovascular properties as well as suppress HPA axis activation via negative feedback (Lee et al., 2015; Jansen et al, 2015; Smith & Vale, 2006). Under acute stress, surges of cortisol can

be beneficial for adjusting to the stressful stimuli (Lee et al., 2015; Jansen et al., 2015; McEwen, 2008). Though moderate amounts of cortisol have potential benefits for completing cognitively demanding tasks (Kukolja et al., 2008), acute sleep deprivation can disrupt HPA activity, potentially disturbing cyclic cortisol secretion (Balbo et al., 2010). Given that cortisol and heart rate responses are both physiological markers of stress, it is necessary to study the connection between cortisol and heart rate reactivity in acute sleep deprived individuals.

**Cortisol and Heart Rate Reactivity**

In stress research on ANS activity mediation, it is imperative to include cortisol as a biomarker of stress. Salivary cortisol peaks generally occur ten minutes after acute psychosocial stress exposure (Dickerson & Kenny, 2004; Rimmele et al., 2007). It appears that while salivary cortisol tends to be elevated after a stressor, average heart rate has been known to be elevated during a stressor (Allen et al., 2014). However, due to individual variability, these findings are not necessarily conclusive.

Work by Rimmele and colleagues (2007) found that athletic individuals had lower cortisol and heart rate reactivity responses to the TSST. On the contrary, De Vente and colleagues (2003) found that heart rate and cortisol reactivity are unaffected by the same psychosocial stressor, regardless of health status. Given that exercise health status may not be enough to impact both cortisol and heart rate reactivity, it is possible that these changes are predominantly influenced by the stressor itself. For instance, both pregnant women and non-pregnant women have been found to have heightened delta heart rate and cortisol reactivity in response to public speaking tasks (De Weerth et al., 2007). In another study, Kunz-Ebrecht and colleagues (2003) found that in a sample of middle-aged White participants, individuals who had higher cortisol responsivity to a virtual version of the Stroop Color and Word task and Mirror Tracing Task also had

lower HRV compared to participants who had lower cortisol responsivity. Despite inconsistent outcomes, research suggests a predictive relationship between cortisol and heart rate reactivity, given that the task is considered highly stressful for the individual (Looser et al., 2010). Therefore, when investigating the effects of acute stress, it is essential to be mindful of individual variability and choose an appropriate stressor to be implemented during experimentation.

Regarding HRV, Schwarz and colleagues (2018) subjected participants to 24 hours of sleep deprivation. Although they found baseline salivary cortisol to be higher in the sleep deprived individuals, HRV and cortisol reactivity were not impacted during or after the TSST. This implied that acute sleep deprivation did not affect cardiovascular responsiveness (Schwarz et al., 2018). However, research using real-life stressors involving medical interns (Tobaldini et al., 2013) and medical residents (Morales et al., 2019) working one night on-call has found that total sleep deprivation combined with work stress reduces HRV, although cortisol reactivity either increased (Morales et al., 2019) or remain unchanged (Tobaldini et al., 2013). These studies suggest that real-life stressors are more effective in triggering robust ANS and HPA responses than laboratory stressors. Nonetheless, research has found that acute sleep deprivation and stress can generate similar physiological symptoms (e.g. elevated heart rate and cortisol secretion) in humans. Thus, it is imperative to further investigate how cortisol and heart rate reactivity are modulated by acute psychosocial stress following a night of total sleep deprivation.

## Chapter Summary

Both acute sleep deprivation and acute stress lead to changes in physiological and cognitive functioning. While previous research has explored the physiological responses (e.g., heart rate reactivity, cortisol reactivity) of acute psychosocial stress and acute sleep

deprivation, they primarily focused on either stress or sleep independently of one another. Therefore, the combined effects of acute sleep deprivation and stress on heart rate reactivity should be further explored. The overarching aim of the current study was to investigate how the combination of acute sleep deprivation and acute psychosocial stress impacts heart rate reactivity.

Aim 1 of the current study examined the impact of acute sleep deprivation on delta heart rate. It was hypothesized that sleep deprived participants would exhibit reduced delta heart rate during an acute psychosocial stressor, the TSST, as compared to control participants. Aim 2 of the current study investigated whether acute sleep deprivation impacted heart rate recovery after an acute stressor. It was hypothesized that sleep deprived participants would have delayed heart rate recovery immediately after an acute psychosocial stressor, as compared to control participants. Lastly, Aim 3 of the present study investigated the relationship between estimated BPM variance and salivary cortisol in acutely stressed-sleep deprived participants and acutely stressed-non-sleep deprived participants through exploratory analyses.

# CHAPTER III:

# METHODS

## Participants

Twenty-eight adults ages of 18-45 years old (*M*=24.68, SD=4.84) participated in the current study. For gender, participants identified as female (57.1%) or male (42.9%). As for race, participants identified as either Hispanic (39.3%), Caucasian (35.7%), Asian (14.3%), Other (7.1%), or African-American (3.6%). Participants were recruited through the UHCL SONA online participant pool, referrals, and flyer advertisements. Participants must have passed the health screening evaluations to be considered eligible for this study. Ineligible participants were those who did not have good health standing, a history of sleep disorders, or a history of mental illness. Anyone living more than thirty minutes away from the campus was not eligible for this study. Participants received monetary compensation and credit towards psychology course assignment requirements via the SONA system.

## Materials

### Trier Social Stress Test

The Trier Social Stress Test (TSST) is a psychosocial stress test that has been shown to elicit acute stress responses from both the HPA axis and sympathetic-adrenal-medulla axis (Bali & Jaggi, 2015; Kirschbaum et al., 1993; Klopp et al., 2012; McRae et al., 2006). During the procedure, a research assistant reads a scenario in which the participant was accused of shoplifting. In the presence of the research assistant, each participant had three minutes to create a defense to the accusation. The researchers informed the participants that they would be recorded and judged by a panel of judges during the interview. Once the three-minute defense preparation ended, the participants were taken into another room where they were instructed to stand and give their speech.

The participants had five minutes to give a speech in their own defense. Afterwards, the participants were instructed to perform a mental arithmetic task for an additional five minutes. During the mental arithmetic math task, participants were asked to subtract 13 from 1,022 and continue for each subsequent answer. If the participant provided an incorrect answer, the interviewer would instruct the participant to restart the task from the beginning. The participants were instructed to continue the mental arithmetic task until the end of the five-minute period.

## Measures

### Self-Reported Mood Scales

The participants completed three surveys relevant to anxiety, stress, and mood: Social Interaction Anxiety Scale, Perceived Stress Scale, and Positive and Negative Affect Scale. The Social Interaction Anxiety Scale (SIAS) is a 20-item questionnaire (Mattick & Clarke, 1998). SIAS measurements are meant to reflect participants' self-reports of anxiety during social interactions. Another scale, the Perceived Stress Scale (PSS), is a 14-question survey which assesses self-reported recent life event stress (Cohen et al., 1983). The third measurement is the Positive and Negative Affect Scale (PANAS): a 20-item questionnaire that primarily measures positive and negative affect that the participants are feeling at the moment (Watson et al., 1988).

### Sleep Quality and Sleepiness Scales

Participants also completed one sleep quality survey and three sleepiness questionnaires. The sleep quality survey completed by participants was the Pittsburgh Sleep Quality Index (PSQI), a 19-question form that assesses an individual's habitual sleeping behaviors (Buysse et al., 1989). Subjective sleepiness was measured through the Epworth Sleepiness Scale (ESS), the Stanford Sleepiness Scale (SSS), and the Karolinska Sleepiness Scale (KSS). Although all three scales measure sleepiness, the ESS is an 8-

item survey that uses daytime scenarios to measure general fatigue and sleepiness (Johns, 1991). The KSS analyzes subjective drowsiness throughout the day, and individuals are to use a scale from one-to-seven to report levels of sleepiness (Hoddes et al., 1973). Similarly, the KSS uses a 9-point Likert scale to evaluate individual tiredness (Åkerstedt & Gillberg, 1990).

**Delta Heart Rate**

Beats-per-minute (BPM) recordings were captured through the Heart Rate & Pulse Logger sensor NUL-208. Heart rate collection lasted ten minutes per time point. Given that delta heart rate is the change in heart rate from a relaxed body position to a standing position, delta heart was calculated by subtracting relaxed (e.g. sitting, lying down) heart rate from standing heart rate. In the current study, BPM recordings for delta heart rate were collected during pre-TSST and TSST time points. Participants sat through pre-TSST recordings, and stood during TSST recordings. Delta heart rate was calculated by subtracting the average heart rate during the first two minutes of the pre-TSST (sitting) from the average heart rate during the first two minutes of the TSST (standing).

**Estimated BPM Variance**

As a proxy for HRV, standard deviations for each participant were calculated as an estimate of beats per minute (BPM) variance at two time points: pre-TSST and TSST. To calculate estimated BPM variance, each participant's average BPM was broken down to analyze average heart rate per second across a 120 second (two minute) interval. Average BPM per second was subtracted from average heart rate of the following second (e.g., second 2 HR – second 1 HR; second 3 HR – second 2 HR; etc.) to find differences in heart rate between each second. Then, the standard deviation of these differences in heart rate was calculated and used as estimated BPM variance.

**Heart Rate Recovery**

For heart rate recovery, BPM recordings were also captured through the Heart Rate & Pulse logger sensor NUL-208. Heart rate was recorded at three time points: pre-TSST, TSST, and immediately post-TSST. These heart rate changes were used to compare average BPM immediately before the TSST (pre-TSST), actively during the TSST, and immediately after the TSST (post-TSST) in both the experimental and control groups. The post-TSST time point was considered the recovery phase. Heart rate collection lasted ten minutes per time point. Research suggests that parasympathetic reactivity occurs in the initial 30 seconds of heart rate recovery (Imai et al., 1994). Cincin and colleagues (2015) have found significant changes in heart rate during the initial 30 seconds and during the initial one minute of recovery (Cincin et al., 2015). Moreover, work by Mezick and colleagues (2014) has reported significant changes in heart rate recovery during the initial two minutes period of recovery (Mezick et al., 2014). Given these findings, the present study analyzed heart rate differences during the time intervals of 30 seconds, one minute, and two minutes. That is, average heart rate during the initial 30 seconds of baseline (Pre-TSST) was compared to average heart rate during the initial 30 seconds of the TSST and average heart rate during the initial 30 seconds post-TSST. Subsequently, changes in heart rate during the initial 60 seconds (one minute) and initial 120 seconds (two minutes) were also compared for each time point.

**Cortisol**

Salivary samples were collected from participants at three time points: pre-TSST, immediately after the TSST, and 20 minutes post-TSST. To collect salivary samples, participants were instructed to place a cotton swab between their lower lip and gum for two minutes. All samples were placed into individual tubes and stored at -20°C for safe keeping. Human enzyme immunoassay kits were used to extract and quantify cortisol

(Salimetrics LLC, USA) derived from the salivary samples. Cortisol was measured in nmol/L. Area under the curve with respect to ground ($AUC_g$) was calculated and used as a global measure of cortisol released during the stressor (Lupien, 2013). $AUC_g$ is often used as an overall measure of cortisol as it gives an estimate of how much cortisol is secreted over a given period of time (Lupien, 2013). For example, Klinkenberg and colleagues (2009) used AUC response curves to investigate the relationship between cortisol and heart rate variability during the TSST. $AUC_g$ takes into account changes in cortisol between samples across a given period of time (Fekedulgen et al., 2007; Pruessner et al., 2003). For each participant, two trapezoidal areas were calculated based on the three cortisol values and the time interval between each measurement. The time interval between the first and second salivary sample was 13 minutes, and the time between the second and third salivary sample was 10 minutes. In total, area under the curve was calculated for cortisol response over the course of 23 minutes.

**Experimental Design**

After recruitment, participants were randomly assigned to one of two groups: the control group or the sleep deprived (experimental) group. The researchers acquired informed consent from participants prior to the start of the experiment, and participants were screened immediately before experimentation to verify that they were still eligible for the study. The participants in the experimental group arrived at the laboratory between 9:00 and 10:00 pm. Prior to arriving in the laboratory, participants were instructed to refrain from consuming caffeine and napping during the 24 hours prior before the study and throughout the duration of the study. After obtaining their informed consent, participants then filled out multiple self-report surveys. The first sets of surveys completed by the participants were three self-reported mood scales: Social Interaction Anxiety Scale (SIAS), Perceived Stress Scale (PSS), and Positive and Negative Affect

Scale (PANAS). Then, the participants completed one sleep quality survey: Pittsburgh Sleep Quality Index (PSQI). Lastly, participants completed three sleepiness scales: Epworth Sleepiness Scale (ESS), Stanford Sleepiness Scale (SSS), and Karolinska Sleepiness Scale (KSS). All surveys were taken immediately before the sleep deprivation session. At the start of the experiment (between 9:00 and 10:00pm), each participant was given a Fitbit to monitor cardiovascular responses throughout the night.

Participants in the experimental group were closely monitored throughout the night by researchers in the lab to ensure wakefulness. Non-stimulating activities, such as using personal electronic devices, drawing, reading, and board games, were used to maintain wakefulness. After eight hours of sleep deprivation, the experimental group underwent an acute psychosocial stressor task, the Trier Social Stress Test, at approximately 7:00 am the following day. Participants had to cease consumption of food and non-caffeinated and non-alcoholic beverages two hours prior to the stressor.

Once experimentation continued the next morning, heart rate, saliva samples, and the PANAS were collected as baseline measurements. As the PANAS is susceptible to mood changes during shorter time intervals, the survey was administered three times: before the TSST (baseline), immediately after the TSST, and 10-minutes post-TSST (recovery). Heart rate measurements were collected continuously at baseline, continuously during the TSST, and immediately after the TSST (recovery). Additionally, salivary samples were collected at the start of baseline, immediately after the TSST, and 10 minutes post-TSST.

Procedures for the control group were the same as those for the experimental group. However, instead of being asked to stay awake for 24 hours, participants in the control condition were instructed to go home that night and sleep immediately to ensure a minimum of eight hours of sleep. Excluding showers, participants were told to not take

off the Fitbit throughout the night and the following morning. They were also instructed to notify the researchers at what time they went to sleep and at what time they woke up. The control participants then came back the following morning at 7:00 am to participate in the TSST.

## Data Analysis

To compare baseline measurements between control and experimental participants, Independent t-tests and Chi-Square tests were conducted. For Aim 1 of the current study, an independent subjects t-test was used to test the hypothesis that delta heart rate between sleep deprived and control participants would differ during the TSST. Additionally, a 2(time)x2(condition) mixed model ANOVA was used to analyze estimated BPM variance during the first two minutes of the TSST as compared to baseline. Estimated BPM variance was calculated for further examination of delta heart rate. In Aim 2, a 3(time)x2(condition) mixed model ANOVA was performed to test the hypothesis that changes in heart rate recovery between sleep deprived and control participants would differ after the stressor. Finally, in Aim 3, a between subjects t-test was used to compare cortisol reactivity between conditions. Additionally, Pearson's correlation coefficients were used to examine the relationship between cortisol reactivity and estimated BPM variance during pre-TSST and TSST time points in both acutely sleep deprived and non-sleep deprived participants.

# CHAPTER IV:

# RESULTS

## Baseline

Twenty-eight participants were recruited for the current study. Due to technical errors, twelve participants were excluded due to loss of heart rate data. Additionally, one participant was excluded for taking micronaps during the sleep deprivation period. This left a total of 15 participants eligible for inclusion in the current analyses. After checking for assumption violations, there were no violations that occurred in the present analysis. Between subjects t-tests were used to compare baseline measurements between conditions. Baseline measurements did not significantly differ between sleep deprived and non-sleep deprived participants in gender, $\chi_2(1)=0.045$, p=0.833, age, $t(13)=-0.128$, $p=0.900$, Epworth Sleepiness Scale, $t(13)=1.881$, $p=0.083$, Karolinska Sleepiness Scale, $t(13)=-1.287$, $p=0.221$, Stanford Sleepiness Scale, $t(13)=-0.955$, $p=0.357$, PSS $t(13)=-0.494$, $p=0.630$, PSQI, $t(13)=1.011$, $p=0.330$, SIAS, $t(13)=-1.275$, $p=0.225$, or heart rate, $t(13)=-1.43$, $p=0.176$ (see Table 1). Although research has found that age and gender may contribute to heat rate and cortisol reactivity (Kudielka et al., 2004), the current study did not control for these variables as there were no significant differences found at baseline.

*Table 1*

*Mean and Standard Deviation Baseline Data per Condition*

| Variable | Control | | Experimental | |
|---|---|---|---|---|
| Gender | | | | |
| Female | 3 | | 4 | |
| Male | 3 | | 5 | |
| | **Mean** | **SD** | **Mean** | **SD** |
| Age | 23.83 | 2.86 | 23.66 | 2.18 |
| PSS | 13.50 | 4.37 | 12.40 | 3.84 |
| PSQI | 4.67 | 1.97 | 6.00 | 2.78 |
| Epworth Sleepiness scale | 5.00 | 2.68 | 7.33 | 2.12 |
| Karolinska Sleepiness Scale | 4.00 | 2.28 | 2.89 | 1.05 |
| Stanford Sleepiness Scale | 2.83 | 1.17 | 2.33 | 0.86 |
| Heart Rate | 73.20 | 4.56 | 66.70 | 10.5 |

## Delta Heart Rate

After checking for normality using Levene's test for equality of variance, ($F$=1.80, $p$=0.203), a between subjects t-test was performed with condition as a between subject factor and delta heart rate as the dependent variable. There was not a significant difference between experimental and control conditions $t$(13)=0.807, $p$=0.434. Given the results, acute sleep deprivation did not have an effect on delta heart rate.

## Estimated BPM Variance

A total of 15 participants were included in the estimated BPM variance analysis. A 2(condition) x 2(time) mixed model ANOVA was performed with condition (sleep deprived or non-sleep deprived) as a between subject factor and time (initial 120 seconds of the pre-TSST and initial 120 seconds of the TSST) as a within subjects factor. There was no interaction effect for condition x time $F$(1,13)=0.040, $p$=0.845. There was a significant main effect for condition $F$(1,13)=5.540, $p$=0.035, indicating that there was a significant difference in estimated BPM variance between sleep deprived and non-sleep deprived individuals. A post-hoc comparison revealed that estimated BPM variance in the control condition was significantly higher than estimated BPM variance in the experimental condition $t$(13)=-2.09, $p$=0.035. This result suggests that non-sleep deprived individuals had a higher magnitude of changes in estimated BPM variance than sleep deprived individuals (see Figure 1).

There was also a main effect for time $F$(1,13)=9.990, $p$=0.008, indicating that estimated BPM variance was significantly different between the pre-TSST and TSST time points. Another post-hoc comparison found that estimated BPM variance was different between pre-TSST and TSST time points $t$(13)=-3.16, $p$=0.008. This result indicates that estimated BPM variance increased during the TSST (see Figure 2).

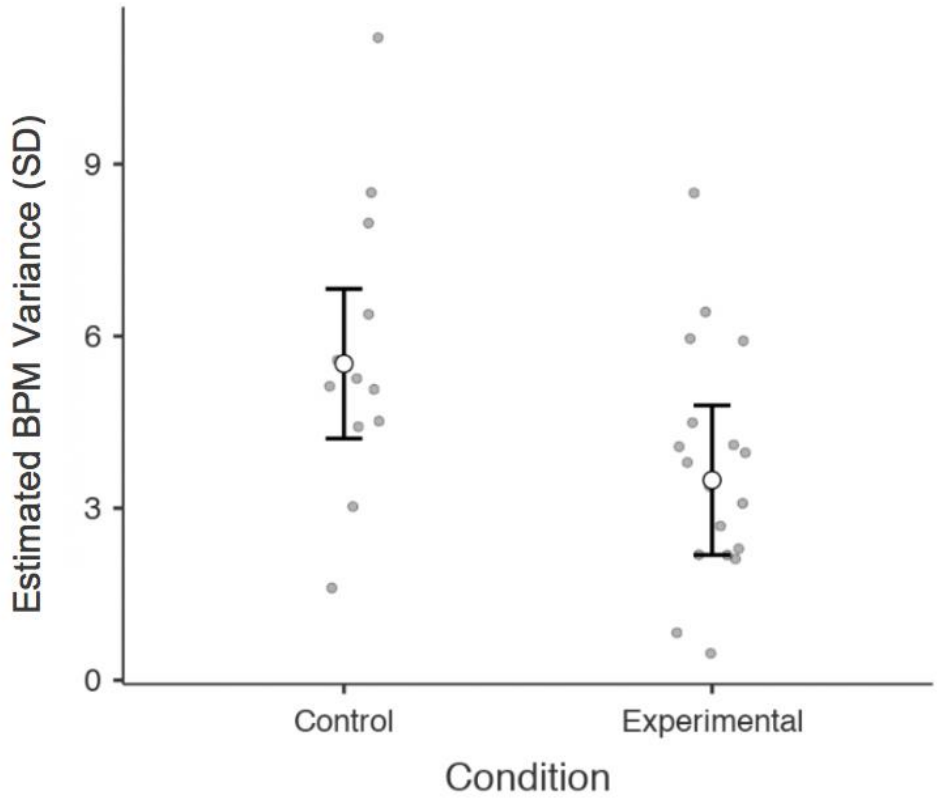


*Figure 1*
*Estimated Marginal Means of Estimated BPM Variance per Condition*

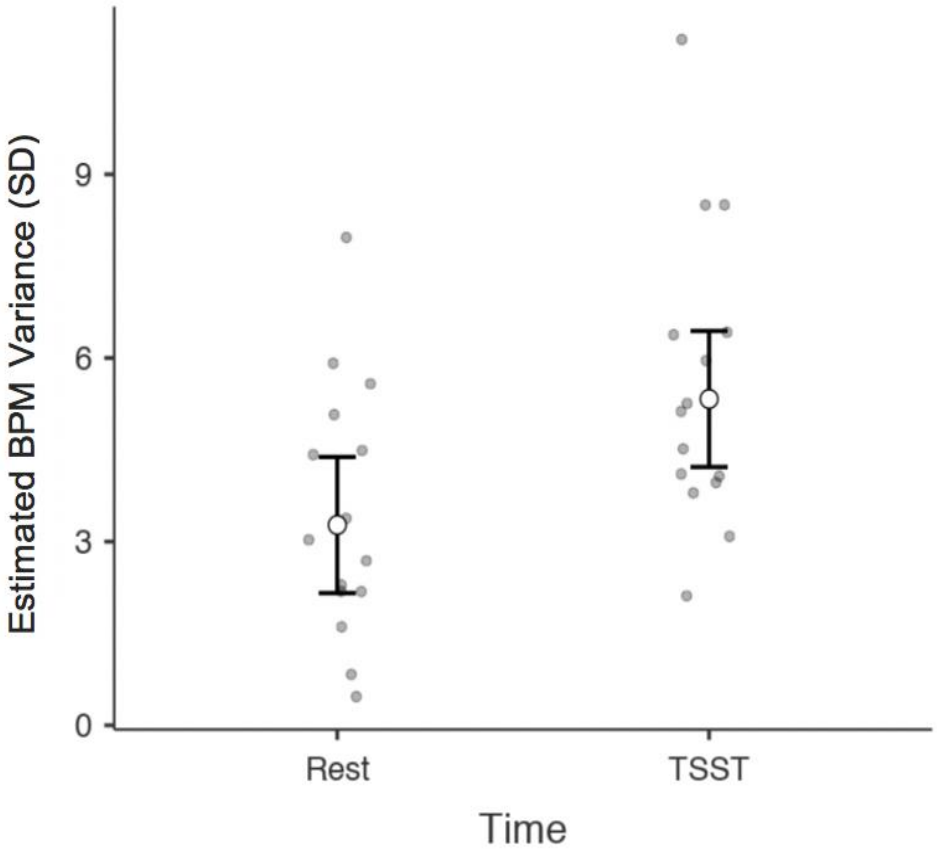


*Figure 2*
*Estimated Marginal Means of Estimated BPM Variance per Time*

**Heart Rate Recovery: Initial 30 Seconds**

A 3(time)x2(condition) mixed model ANOVA was performed with condition as a between subject factor and heart rate in the initial 30 seconds of each time point (pre-TSST, TSST, and post-TSST) as repeated measures. Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $W$=0.727, $p$ = 0.148 Table 2 summarizes the results of the mixed-model ANOVA analysis. There was not an interaction effect for condition x time. There was not a significant main effect for condition. Additionally, there was not a significant main effect for time.

*Table 2*

*Initial 30 Seconds HR Repeated Measures ANOVA*

| Effect | *MS* | *df* | *F* | *p* |
|---|---|---|---|---|
| Condition | 5.08 | 1 | 0.030 | 0.866 |
| Time | 4.83 | 2 | 0.038 | 0.963 |
| Condition x Time | 83.31 | 2 | 0.647 | 0.532 |
| Residual | 128.85 | 26 | | |

## Heart Rate Recovery: Initial 60 Seconds

A 3(time)x2(condition) mixed model ANOVA was performed with condition as a between subject factor and heart rate in the initial 60 seconds of each time point (pre-TSST, TSST, and post-TSST) as repeated measures. Table 3 summarizes the results of the mixed-model ANOVA analysis. Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $W = 0.734$ $p = 0.156$ There was not an interaction effect for condition x time. There was not a significant main effect for condition, and there also was not a significant main effect for time.

*Table 3*

*Initial 60 Seconds HR Repeated Measures ANOVA*

| Effect | *MS* | *df* | *F* | *p* |
|---|---|---|---|---|
| Condition | 83.50 | 1 | 0.599 | 0.453 |
| Time | 84.30 | 2 | 1.055 | 0.362 |
| Condition x Time | 23.70 | 2 | 0.297 | 0.745 |
| Residual | 79.90 | 26 | | |

## Heart Rate Recovery: Initial 120 Seconds

A 3(time)x2(condition) mixed model ANOVA was performed with condition as a between subject factor and heart rate in the initial 120 seconds of each time point (pre-TSST, TSST, and post-TSST) as repeated measures. Table 4 summarizes the results of the mixed-model ANOVA analysis. Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $W = 0.673$, $p = 0.093$ There was not an interaction effect for condition x time. There was not a significant main effect for condition, and there also was not a significant main effect for time.

*Table 4*

*Initial 120 Seconds HR Repeated Measures ANOVA*

| Effect | *MS* | *df* | *F* | *p* |
|---|---|---|---|---|
| Condition | 184 | 1 | 1.52 | 0.239 |
| Time | 55.80 | 2 | 0.727 | 0.493 |
| Condition x Time | 47.80 | 2 | 0.623 | 0.544 |
| Residual | 76.70 | 26 | | |

## Cortisol

Measurements of cortisol production and sensitivity were calculated for each participant by using area under the curve in respect to ground ($AUC_g$). A total of 15 participants were included in the cortisol analysis. After checking for normality using Levene's test for equality of variance, ($F=1.80$, $p=0.203$), a between subjects t-test was used to compare cortisol $AUC_g$ between conditions. There was not a significant difference in cortisol $AUC_g$ between control and experimental participants $t(13)=-0.466$, $p=0.649$, indicating that cortisol reactivity was the same between sleep deprived and non-sleep deprived participants.

**Estimated BPM Variance and Cortisol**

As significant between-group differences in heart rate reactivity were only observed in measurements of estimated BPM variance, the current exploratory analyses further investigated the relationship between cortisol reactivity and estimated BPM variance. Pearson's correlation coefficients were used to examine the relationship between cortisol $AUC_g$ and estimated BPM variance during pre-TSST (SD REST) and TSST (SD TSST) time points. There were not any significant relationships found between cortisol $AUC_g$ and estimated BPM variance during pre-TSST ($r$=0.206, $p$=0.461) and TSST ($r$=0.383, $p$=0.159) time points.

Additionally, Pearson's correlation coefficients were also used to examine the relationship of cortisol reactivity and the average estimated BPM variance across the pre-TSST and TSST periods (SD AVG). There also was not a significant relationship found between cortisol $AUC_g$ and average estimated BPM variance across the two time points, $r$=0.356, $p$=0.192 (see Figure 3).

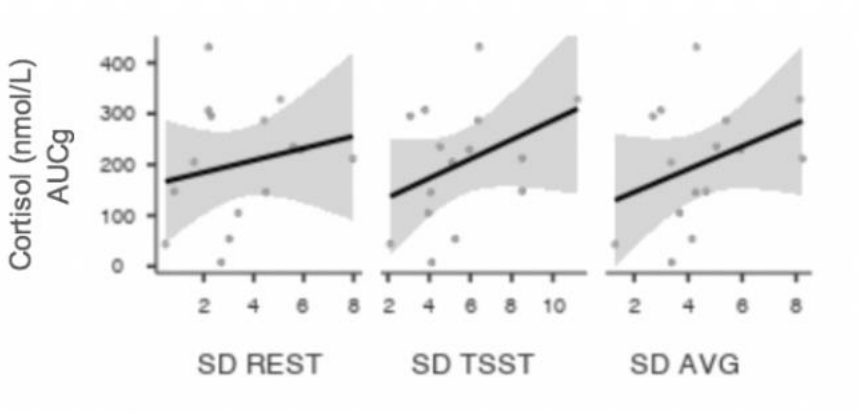


*Figure 3*
*Cortisol Reactivity (nmol/L) in Relationship to Estimated BPM Variance*

# CHAPTER V:

# DISCUSSION

The present study investigated the effects of acute sleep and stress on heart rate reactivity. Acutely sleep deprived and non-sleep deprived participants were all exposed to the TSST. Heart rate reactivity measurements were examined through delta heart rate, estimated BMP variance, and heart rate recovery. Additionally, the relationship between heart rate reactivity and cortisol reactivity was also explored. Overall, the current study found that sleep deprivation had minimal effects on heart rate reactivity during an acute stressor as evidenced by differences in estimated BMP variance. Acute sleep deprivation and stress, however, did not affect delta heart rate, heart rate recovery, or cortisol reactivity.

The aim of the first research question was to investigate whether or not acute sleep deprivation has an effect on delta heart rate during an acute psychosocial stressor. The first hypothesis was not supported by the results in the present study. As delta heart rates were not significantly different between conditions, the results suggest that a single night of total sleep deprivation in combination with an acute psychosocial stressor does not impact delta heart rate. These findings are inconsistent with studies that report delta heart rate increases after public speeches (De Weerth, 2007; Lennartsson et al., 2012) and decreases after acute sleep deprivation (Tobaldini et al., 2013). Given that delta heart rate has been primarily been utilized in exercise research (Girotra et al., 2012; Gorelik et al., 2006; Swaim 2012), it is possible that a psychosocial stressor, such as the TSST, is not effective enough to impact delta heart rate in comparison to physical stressors. The present findings also indicate acute sleep deprivation did not impact delta heart rate. Although people who suffer from obstructive sleep apnea tend to have lower delta heart rate than healthy people without chronic sleep disorders, there are so few studies that

have examined delta heart rate in healthy, acutely sleep deprived individuals (Tobaldini et al., 2013; Tobaldini et al., 2014) that it is difficult to form a conclusion. With minimal research available on the relationship between delta heart rate and sleep, the effects of acute stress on delta heart rate on sleep deprived individuals remain inconclusive.

Further analysis between pre-TSST and TSST time points found that there were significant differences in estimated BPM variance. Although HRV measures the variation of time in between heartbeats, estimated BPM variance was used as proxy for HRV to explore how much heart beats deviated from average heart rate per second. Non-sleep deprived participants had significantly higher estimated BPM variance than sleep deprived participants. Throughout experimentation, sleep deprived participants had blunted variation in their heart beats throughout a two-minute interval in comparison to non-sleep deprived participants. Results of the estimated BPM variance partially support previous HRV research that has found acute sleep deprivation and partial sleep restrictions to be associated with reductions in HRV (Morales et al., 2019; Tobaldini et al., 2013; Wehrens et al., 2012). However, HRV measurements are preferred over estimated BPM variance as HRV frequency domain analysis can evaluate attenuation of ANS subdivisions.

The results of the present study also do not support the second hypothesis which stated that acute sleep deprived participants would exhibit delays in heart rate recovery after an acute stressor. Comparing heart rate during the initial 30 seconds, initial 60 seconds, and initial 120 seconds of all three time points revealed that there were not any significant differences in BPM for either condition. This indicated that heart rate recovery after the TSST was unaffected in both sleep deprived and non-sleep deprived participants. Whereas previous research has found that heart rate recovery became delayed after acute stress (Verkuil et al., 2014) and acute deprivation (Yang et al., 2012),

the current study was unable to find any changes in heart rate during pre-TSST, TSST, and post-TSST time points.

Similar to the findings from Kato and colleagues (2000), heart rate remained unaffected by acute sleep deprivation. However, as the TSST is an established psychostressor known to accelerate heart rate (Boesch et al., 2014), the inability to find significant changes in heart rate in the present study may be contributed to user-error of the NUL-208. Talking during cardiovascular measurement increases blood pressure and heart rate (Liehr, 1992; Zheng et al., 2011). Research has also found that SNS activity increases during read aloud (Dodo & Hashimoto, 2019) and verbal description tasks (Arnold et al., 2014). Given that participants were permitted to engage in conversation and make small movements during heart rate collections, heart rate recording during the rest (pre-TSST) and recovery (post-TSST) time points may not have reflected the participants' true resting heart rates.

Lastly, there were no significant relationships found between cortisol and estimated BPM variance. Cortisol reactivity remaining unaffected by a night of total sleep deprivation is consistent with previous research (Donald et al., 2017; Follenius et al., 1992; Salin-Pascual et al., 1998). However, it also contradicts research that has found baseline cortisol to be higher in acutely sleep deprived individuals (Schwarz et al, 2018; Wright et al., 2015). Cortisol is predominantly determined by changes in diurnal rhythm (Oster et al., 2017). Therefore, saliva collection timing in the present study may have affected cortisol reactivity. After the first night of data collection, the second half of the experiment began at 7:00 am the following morning. The first salivary sample was collected approximately 15 minutes after the start of the experiment. If morning cortisol peaks occur around 8:00 am (Oster et al., 2017), then it is possible that morning cortisol levels were still surging after the sample collections. The second saliva sample was

collected approximately 28 minutes after the start of the experiment, and the third saliva sample was collected approximately 38 minutes after the start of the experiment. This meant that samples were collected across an interval of 23 minutes. Due to cortisol peaks generally occurring in the early mornings and gradually declining throughout the day (Chan & Debono, 2010), it is possible that cortisol responsiveness to the TSST was masked by diurnal cortisol that was still increasing to morning peak levels.

With estimated BPM variance and cortisol rendering no significant relationship in either condition, these results parallel similar findings in studies that have used heart rate reactivity measurements, such as HRV (Schwarz et al., 2018; Tobaldini et al., 2013) and heart rate (De Vente et al., 2003), to evaluate acute stress. Taken together, the results of the present study indicated that an acute psychosocial stressor combined with acute sleep deprivation was not impactful enough to activate significant autonomic responses. Excluding methodology, it can be speculated that individual variability contributed to the apparent lack of relationship between cortisol and estimated BPM variance. For both the HPA and ANS responses to act in synchrony, the task must be highly stressful for the individual (Looser et al., 2010). PANAS surveys were collected from each participant to assess stress levels by time point, but they were not reported in the analyses as they are beyond the scope of the present study. Although the TSST has been known to elicit both cortisol and heart rate reactivity, some participants may have found the stressor to be to be mild.

## Study Limitations

There were multiple limitations throughout the study. Due to the heart rate application crashing multiple times, much of the heart rate data was either lost or not continuously recorded throughout the intended ten-minute intervals. Twelve participants were not included in the analyses of the present study as their heart rate measurements

were missing in one or more (pre-TSST, TSST, or post-TSST) time points. Another technical error that occurred throughout the study was that the Fitbits given to each participant had failed to record nightly physiological activity. Given that control participants were allowed to go home, the researchers relied only on self-reported sleep quality and quantity surveys completed by the controls.

A major limitation of the present study was that the PSS survey and sleepiness surveys were only collected during the first night of experimentation. As the participants had completed the self-reported surveys before they underwent sleep deprivation and the stressor, it is impossible to interpret the relationship of subjective sleepiness and stress levels with heart rate and cortisol reactivity. Additionally, physiological responses were only collected the following morning of the experiment. Thus, in future studies, comparing cortisol and heart rate reactivity to baseline measurements prior to the eight-hour sleep deprivation period would yield more definitive results.

Another limitation was the small sample pool of participants. Only 15 participants were included in the present study and the small sample size may have reduced statistical power in the analyses. There were also occasions where the research assistant who monitored a sleep deprived participant was also the one who conducted the TSST interview for that participant. As research assistants and sleep deprived participants were in the same room for 8 hours, the participants would have become acclimated to the research assistant. Familiarity with the interviewer could have potentially diminished the effects of the TSST. Also, due to user error on the researchers' part, heart rate was collected in BPM instead of arbitrary units. This made it difficult for an HRV analysis as times were set to one second intervals. Thus, estimated BPM variance was used in proxy for HRV. Unfortunately, this meant that estimated BPM variance could not be used to assess PNS and SNS activity.

## Suggestions for Future Research

The present study contributes to a growing body of research that suggests that acute sleep deprivation and stress impacts some level of heart rate reactivity. Future studies should measure the participant's heart rate reactivity before subjecting them to acute sleep deprivation. Given that the present study revealed sleep deprived participants to have lower estimated BPM variance than non-sleep deprived participants, it is evident that heart rate reactivity was affected by sleep deprivation. However, to better understand how acute sleep deprivation impacts heart rate reactivity, heart rate post-sleep deprivation should also be compared to heart rate pre-sleep deprivation. Another suggestion for future research is to use HRV instead of estimated BPM variance. HRV frequency domain metrics allow researchers to locate frequency bands of interest. Thus, utilizing HRV in future studies would provide better insight of PNS and SNS activity.

Individual variability such age and gender should be considered in future research. Research has found that factors such as age and gender influences heart rate reactivity (Kudielka et al., 2004). Additionally, future research should further analyze participants' social interaction histories. Negative social interaction has been associated with reductions of HRV (Shahrestani et al., 2015), and HRV has also been found to become blunted in highly anxious people during high self-disclosure conversation (Ketay et al., 2018). Lastly, a larger sample size of at least 30 participants are recommended for future studies to improve power levels.

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