THE IMPACT OF SLEEP DEPRIVATION AND STRESS REACTIVITY ON INTERLEUKIN-6

by

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Dedication

For Logan, Talitha, and Ragnar, who have always been by my side, even though they will never be able to read this.

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ABSTRACT

THE IMPACT OF SLEEP DEPRIVATION AND STRESS REACTIVITY ON INTERLEUKIN-6

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Interleukin-6 (IL-6) has been implicated as a biomarker for both stress and sleep deprivation. Gaining a clearer understanding of the impact of both conditions concurrently may provide a better understanding of the biological basis of both sleep and stress. We developed an experiment that combined sleep deprivation and the Trier Social Stress Test in order to see how the two conditions interact. We found no significant impact of stress and sleep deprivation on IL-6 levels. Our findings indicate that more research must be conducted in order to fully test this relationship.

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CHAPTER I:

INTRODUCTION

Context of the Problem

Sleep has been shown to be beneficial to human health and wellbeing, allowing for better emotional regulation (Vandekerckhove & Cluydts, 2010), better ability to work on tasks and retain focus (Engle-Friedman, 2014), a lowered propensity for risk-taking (Short & Weber, 2018), and a lessened susceptibility to conditions such as obesity, diabetes, and hypertension (Goel, Basner, Rao, & Dinges, 2013). The American Academy of Sleep Medicine (AASM) and the Sleep Research Society (SRS) recommend at least seven hours of sleep per night for optimal health (N. Watson et al., 2015). Even a single night of sleep deprivation is comparable to long-term sleep restriction (Rupp, Wesensten, & Balkin, 2012) and can show interruption in sustained attention, slowed reaction times, reduced cognitive processing speed, and an increase in reported sleepiness (Frenda & Fenn, 2016). Sleep deprivation is a public health concern, as it has been linked to several illnesses as well as impaired cognitive functioning, especially in terms of error-making and an increased risk of accidents (N. Watson et al., 2015).

Sleep deprivation elicits a psychological and physiological response to stress (N. Watson et al., 2015). This is especially true for those with employment that requires shift work where sleep deprivation is common, and for those routinely required to operate with little sleep and under stressful circumstances, such as the military and first responders (Lincoln, Moore, & Ames, 2018; Parry, Oeppen, Amin, & Brennan, 2018). The impact of sleep deprivation and stress on a person varies, and individual variability could be due to differences in unknown neurophysiological or genetic mechanisms (Van Dongen, Vitellaro, & Dinges, 2005). For individuals with chronic insomnia, there is abnormal hyperactivation during sleep in the hypothalamus, upper brainstem, medial prefrontal

cortex, and amygdala. Interestingly, these regions are known for their actions in the body's arousal/wake system (Saper, 2013). Although studies have shown sleep deprivation lowers the threshold for the subjective perception of stress (Minkel et al., 2012), there has not been, to our knowledge, a study for the biological effects nor on the interaction of both stress and sleep deprivation on other factors.

When it comes to biomarkers of stress and sleep deprivation independently, sleep deprivation has been shown to increase cortisol levels (Leproult, Copinschi, Buxton, & Van Cauter, 1997) and decrease α-amylase levels (Pajcin et al., 2017). Moreover, interleukin-6 (IL-6), a cytokine with many roles in the body including neurogenesis and inflammation (Erta, Quintana, & Hidalgo, 2012), has been found to be sensitive to sleep deprivation and stress. IL-6 has been tested in knockout mice and shown to have some effect on sleep modulation (Morrow & Opp, 2005). IL-6 is sensitive to the pro-inflammatory response promoted by sleep deprivation (Alkadhi, Zagaar, Alhaider, Salim, & Aleisa, 2013) and increases in the presence of socio-cognitive stress (i.e., an academic examination) (La Fratta et al., 2018). While IL-6 is associated with anti-inflammatory properties at an acute stress level, it is associated with pro-inflammatory properties at the chronic level (Gabay, 2006). Given that IL-6 has not been investigated with sleep deprivation and stress concurrently, it is unknown if the socio-cognitive stress increase of IL-6 would be compounded by the presence of sleep deprivation. Further exploration of this relationship could provide more information on the involvement of IL-6 in these responses.

Purpose of the Study

In the current study, we aim to investigate how sleep deprivation affects interleukin-6 (IL-6) production when an individual is exposed to an acute psychosocial stressor (in this case, the Trier Social Stress Test). We hypothesize that individuals who are sleep deprived will show elevated IL-6 levels when exposed to the Trier Social Stress Test as compared to individuals who are not sleep deprived.

CHAPTER II:
REVIEW OF LITERATURE

Sleep Deprivation

The reason why humans sleep is a matter of much debate. Theories range from sleep acting as a process of meta-regulation of many different processes in the body (Vyazovskiy, 2015); as a way to reduce an individual's need for energy during inefficient time periods or to avoid predators; as a time for neural reorganization and changes in the brain's structure and functioning; or as a time period for the body to repair itself, readjust, and return to biological homeostasis (Mignot, 2008; Rasch & Born, 2013), which is further delineated as a way to renormalize synapses after wakefulness has potentiated them (Frank, 2012, 2013).

Sleep itself can be defined as "a natural and reversible state of reduced responsiveness to external stimuli and relative inactivity, accompanied by a loss of consciousness" (Rasch & Born, 2013, p. 681). The two-process model of sleep–wake regulation has been applied to the temporal profiles of sleep (Borbély, 1982; Daan, Beersma, & Borbély, 1984) and daytime vigilance (Achermann & Borbély, 1994). The model consists of a homeostatic process and a circadian process, which combine to determine the timing of sleep onset and offset (Goel et al., 2013).

When the homeostat increases above a certain threshold, sleep is triggered; when it decreases below a different threshold, wakefulness occurs. Sleep in mammals consists of two core sleep stages: slow-wave sleep (SWS) and rapid-eye-movement (REM) sleep, which alternate in a cyclic manner, and are accompanied by two lighter non-REM stages, N1 and N2, which occur during the transitions between SWS and REM, and from wake to sleep and vice versa. In human nocturnal sleep, SWS is most prevalent during the early part of sleep and decreases in intensity and duration across the sleep period, while REM

sleep becomes more protracted and intense towards the end of the sleep period. SWS is identifiable by slow high-amplitude EEG oscillations, whereas REM sleep is distinguishable by wakelike fast low-amplitude oscillatory brain activity (Rasch & Born, 2013).

Benefits of Sleep

For all the debate on the subject, this much is clear: humans engaging in sleep is beneficial and has been linked to better emotional regulation (Vandekerckhove & Cluydts, 2010), better ability to work on tasks (Engle-Friedman, 2014), higher levels of attention and cognitive performance, and a lessened propensity to conditions such as obesity, diabetes, and hypertension (Goel et al., 2013).

Specific portions of sleep benefit certain aspects of life. For example, there is a beneficial effect of SWS-rich early sleep on declarative memory, and a beneficial effect of REM-rich late sleep on nondeclarative memory (Rasch & Born, 2013). Much of this research is based on the 'night-half paradigm,' which splits the sleep phase into two block of three to four hours, and has participants either learn and then sleep for the second half of the night, or sleep for the first half of the night and then learn. This is due to the fact that early nocturnal sleep contains most of the SWS, while the later nocturnal sleep contains more REM; however, the paradigm has a weakness when accounting for N2 sleep (Rasch & Born, 2013).

Problems with Sleep Deprivation

It therefore becomes a problem when individuals lack enough sleep: either in the form of sleep deprivation or sleep restriction, both can cause severe problems (Rasch & Born, 2013). One particular Rechtschaffen and Bergmann (1995) study illustrates the dangers of sleep deprivation when occurring for too long. In that work, rats were sleep deprived using the 'disk over water' method. In summary, when a rat (which has been

placed on a disk suspended over water) begins to enter a particular sleep stage, the disk rotates and the rat must walk to the other portion of the disk to avoid being submerged in the water. The experimenters conducted studies using several different types of sleep deprivation in comparison to controls: total sleep deprivation (where sleep was reduced by 91%), paradoxical sleep deprivation (where REM sleep was reduced by 99% in one cohort and 86% in a second), and high EEG amplitude non-REM (NREM) sleep deprivation (reduced by 96%). In all cases, the non-control rats either died during the experiment or were killed when death was imminent. The mean survival time for total sleep-deprived rats was 2-3 weeks; the first paradoxical sleep-deprived cohort had a mean survival time of 5 weeks, while the second only had a slightly longer mean survival time; the high EEG amplitude NREM sleep-deprived rats had a mean survival of 6.5 weeks (Rechtschaffen & Bergmann, 1995). The study concluded that sleep deprivation can and does significantly shorten life span, and such a major negative impact on longevity should not be treated lightly.

In humans, both physiological and cognitive deficits can be found after sleep deprivation or restriction. An example of immune function impairment is in Cohen, Doyle, Alper, Janicki-Deverts, & Turner (2009), where subjects kept a record of their sleeping patterns for two weeks and were then exposed while under quarantine to a rhinovirus via nasal drops. The study monitored the subjects for five days after viral exposure and found that subjects sleeping less than 7 hours a night had an almost triple chance (2.94 times) of developing a cold than individuals sleeping 8 hours or more (Cohen et al., 2009). Spiegel, Leproult, and Van Cauter (1999) showed both a lowered glucose tolerance and lowered concentrations of thyrotropin (which regulates the thyroid) in subjects who were restricted to 4 hours of sleep for 6 nights, as opposed to fully rested

controls. Additional impairments have been shown in acetylcholine-induced vasodilation, endothelial dysfunction, and arterial stiffness (Sauvet et al., 2017).

Cognitive impairments during sleep deprivation are plentiful. Acute sleep loss, either through sleep deprivation or sleep restriction, leads to individuals performing poorly on tasks related to alertness and sustained attention (Frenda & Fenn, 2016; Ma, Dinges, Basner, & Rao, 2015). In particular, a study by Van Dongen, Maislin, Mullington, and Dinges (2003) explored tasks after chronic sleep restriction of 4 hours, 6 hours, or 8 hours per night for 14 consecutive days, and total sleep deprivation for 3 days. The study found that cognitive and psychomotor performance decreased at a steady cumulative rate in all four cases, with a graded response based on the amount of restriction. Chronic restriction of sleep of 6 hours per night for 14 days produced cognitive performance deficits comparable to those found under conditions of 1 day of total sleep deprivation, and restriction to 4 hours per night for 14 days was comparable to 2 days of total sleep deprivation (Van Dongen et al., 2003).

Behaviorally, humans who are sleep deprived have difficulties with information and skill acquisition, are more likely to repeat failed strategies, and are less likely to adjust strategy in the event of failure (Frenda & Fenn, 2016). Even tasks with which individuals are highly skilled can show deficits related to sleep loss. For example, a study of ten military jet pilots who were sleep deprived for 38 hours showed significant deficits to performance in a flight simulator (Van Dongen, Caldwell, & Caldwell, 2006).

These deficits have been found to persist even through recovery sleep. Axelsson, Kecklund, Akerstedt, Donofrio, Lekander and Ingre (2008) showed that the deficits associated with 5 days of restricted sleep of 4 hours were not fully alleviated even after seven days of recovery sleep. Research has, therefore, looked into napping as a way to combat the negative effects developed during sleep deprivation. Vgontzas et al. (2007)

showed a two hour nap in the afternoon after a night of total sleep loss can significantly restore alertness and slightly improve cognitive performance in healthy adults, though these improvements did not reach baseline.

Shift Work and Sleep

Shift work, including working evenings, nights, or rotating shifts, affects the sleep cycle and level of alertness of working Americans; approximately 15% of full-time workers in the US work shifts that include nights (Goel et al., 2013). This type of shift work is often associated with shorter and more disrupted sleep periods, additional obstructions to circadian phases, sleepiness, and fatigue (Driscoll, Grunstein, & Rogers, 2007; Goel et al., 2013; Vgontzas et al., 1999).

Subjective Measures of Sleepiness and Alertness

There exist many subjective measures of sleepiness and alertness, which tend to only reflect circadian variation in the event the measures ask about the subject's immediate levels of sleepiness and alertness. These measures have many types of rating, including Likert rates (Stanford Sleepiness Scale, Epworth Sleepiness Scale), visual analog scales (Monk, 1989), and adjective checklists such as the Activation-Deactivation Adjective Check List and Profile of Mood States (Goel et al., 2013).

Chronotype, or a person's tendency to stay awake in the early morning or late at night, differs endogenously in the circadian phase of their biological clock. Self-report questionnaires, such as the Horne-Östberg Morningness-Eveningness Questionnaire and the Munich ChronoType Questionnaire, are commonly utilized measures of circadian phase preference. Chronotypes show differences in neurobehavioral responses to sleep fragmentation, total sleep deprivation, and risk-taking propensity at baseline and following total sleep deprivation (Goel et al., 2013). Both age and sex affect morningness-eveningness; children have an earlier chronotype, swiftly becoming later

until 20 years of age, whereupon their chronotype gradually becomes earlier and earlier with age, while women skew towards morningness in comparison to men (Goel et al., 2013; Roenneberg et al., 2007).

The constant routine protocol, a standard to measure circadian rhythmicity, is based on keeping subjects awake in a fixed posture in a constant lab environment for at least 24 hours, with frequent measurements of body temperature, plasma, blood, and saliva to indicate which measurements are under circadian control and independent of sleep (Goel et al., 2013). Overall, body temperature is one of the most important measures of circadian variation and, under entrained conditions, can indicate the cognitive abilities of the subject, with higher body temperature typically corresponding to good cognitive performance. These tasks include search-and-detection tasks, simple and choice reaction time tasks, sorting, logical reasoning, memory access, meter reading accuracy, and school performance. Circadian variation in task performance is enhanced when sleep deprivation occurs, and circadian rhythmicity of neurobehavioral performance covaries with subjective sleepiness scores (Baehr, Revelle, & Eastman, 2000; Goel et al., 2013; Kerkhof & Van Dongen, 1996).

Sleep and Affect

Without enough healthy sleep, negative emotional reactivity seems to be enhanced significantly and positive reactions to positive events often are subdued (Zohar, Tzischinsky, Epstein, & Lavie, 2005). Sleep loss intensified negative emotions and diminished positive emotions following a goal-thwarting or goal-enhancing event (Vandekerckhove & Cluydts, 2010; Zohar et al., 2005). Vandekerckhove and Cluydts (2010) further posit the hypothesis that a diminished control of the prefrontal cortex may enable the brain to increase the processing of emotions related to ongoing or unfinished negative life experiences, all while asleep.

Morning mood improves when REM-sleep is intact but worsens after a night of sleep deprivation (Cartwright, Luten, Young, Mercer, & Bears, 1998; Vandekerckhove & Cluydts, 2010). Deprivation of REM-sleep also reduces functionality of affect regulation (Andreassi, 2010; Vandekerckhove & Cluydts, 2010) in comparison with non-REM-deprived or undisturbed sleep.

Brain Regions and Sleep

Certain brain regions are activated during the different cycles of sleep. During non-REM sleep, regions including the prefrontal cortex, anterior cingulate cortex, thalamus, basal ganglia, precuneus, and hypothalamus have reduced activity (Maquet, 2000; Rasch & Born, 2013). During REM sleep, the hippocampus, amygdala, anterior cingulate cortex, and primary visual cortex have activation similar to non-sleep, while the prefrontal cortex has reduced activity (Rasch & Born, 2013; Vandekerckhove & Cluydts, 2010).

There is a potential neurobiological mechanism to compensate for drowsiness after sleep loss (Goel et al., 2013), which suggests that during sleep deprivation the brain must work harder in order to keep at optimal performance levels (Frenda & Fenn, 2016). During sleep deprivation, reduced activity in the prefrontal cortex is found, even after only 24 hours of continuous wakefulness (Frenda & Fenn, 2016; Ma et al., 2015; Thomas et al., 2000; Vandekerckhove & Cluydts, 2010). Drummond et al. (2000) found an increase in the left prefrontal cortex during encoding of a memory task as well as recruitment of areas not active during a rested state, suggesting a compensatory response to sleep deprivation. In PET scans following 32 hours of sleep deprivation, significantly reduced glucose metabolism occurred in the frontal and temporal lobes, thalamus, basal ganglia, and cerebellum, while reduced glucose metabolism of only the thalamus, basal ganglia, and frontal lobe were found in a 24-hour replication study. In the thalamus,

activity appears to increase following 24-hour sleep deprivation, but not in 36-hour sleep deprivation (Ma et al., 2015). These inconsistencies arise likely due to differing neuroimaging techniques, length of sleep deprivation, and/or type of cognitive tasks.

Stress

Stress is a bodily event where, after an intrinsic or extrinsic force or situation is deemed threatening by the body through a distribution of neural connections, a combination of physiological and behavioral responses occur to combat the stressor; depending on the resources and capability of the individual, the same event that induced stress before may not a second time, or vice versa (Almojali, Almalki, Alothman, Masuadi, & Alaqeel, 2017; McEwen & Gianaros, 2010; Tsigos, Kyrou, Kassi, & Chrousos, 2000). There are several specific requirements a situation must have in order to induce a stress response: these factors are novelty, unpredictability, social evaluation, and/or a lack of control over the situation (Dickerson & Kemeny, 2004; Lupien et al., 2007). In addition, stress can be either absolute or relative. An absolute stressor describes those threats that would lead to a significant stress response which would be required for one's survival or well-being, such as facing a natural disaster, handling extreme heat or extreme cold, or confronting a dangerous animal. A relative stressor, on the other hand, describes stressors that will only elicit a response from a certain proportion of individuals and can have either a mild or pronounced effect, such as a public speaking task (Lupien et al., 2007).

Finally, stress can be acute or chronic. As a temporary measure, the stress response system activates behavioral, physical (Morimoto & Alexopoulos, 2011), antireproductive, antigrowth, catabolic, and immunosuppressive effects (Tsigos & Chrousos, 2002) that perform in a beneficial manner to combat the stressor. Behavioral adaptations during stress include increased arousal, alertness, and vigilance. Physical

adaptations during stress include oxygen and nutrients directed to the central nervous system and stressed body site(s), altered cardiovascular tone, increased blood pressure and heart rate, and increased respiratory rate (Tsigos et al., 2000). This can in some cases be helpful: however, when this system is activated for too long, the stress mechanism becomes dysregulated and its effects become damaging (McEwen & Gianaros, 2010; Tsigos & Chrousos, 2002). In addition, some benefits of acute stress are reversed when the stress becomes chronic -- for example, while acute stress has been found to enhance antiviral defenses (Edwards et al., 2006; Slavich & Irwin, 2014), chronic stress has been associated with reduced antiviral defenses (Slavich & Irwin, 2014).

The stress response itself is mediated by a biological infrastructure located in both the central and peripheral nervous system (Tsigos et al., 2000). The two major physiological pathways for this are the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis (Slavich & Irwin, 2014).

Hypothalamic-Pituitary-Adrenal (HPA) Axis

The major stress axis in humans is the HPA axis. When a stressor is detected, corticotropin-releasing hormone (CRH) is secreted from the paraventricular nucleus of the hypothalamus and travels to the anterior pituitary gland, where it stimulates adrenocorticotropic hormone (ACTH) and releases it into the bloodstream. When ACTH reaches the adrenal cortex, it initiates the synthesis and secretion of glucocorticoids (called corticosterone in animals, and cortisol in humans). Cortisol then targets multiple sites in the body which deal with functions such as metabolism and immune function, mostly to increase energy availability. Cortisol further contributes to its own regulation by binding to key feedback sites, including the pituitary gland, hypothalamus, hippocampus, amygdala, and prefrontal cortex (Dedovic et al., 2009; Herman, Ostrander, Mueller, & Figueiredo, 2005; Vgontzas et al., 2007). The hippocampus, amygdala, and

prefrontal cortex also regulate the HPA axis, with the hippocampus and prefrontal cortex generally being inhibitory and the amygdala being excitatory (Herman et al., 2005; McEwen & Gianaros, 2010). The hippocampus and amygdala process experiences and help to interpret whether an event is threatening or otherwise stressful (McEwen & Gianaros, 2010).

The HPA axis has its own circadian and ultradian rhythm, where a pulse of production and release of glucocorticoids occurs every 1-2 hours (Tsigos et al., 2000).

Certain types of acute stress and chronic stress suppress neurogenesis and cell survival (McEwen & Gianaros, 2010). During acute stress, CRH and arginine-vasopressin (AVP) increase in secretion and frequency, as well as other inflammation markers including cytokines (Tsigos et al., 2000). There is also a decreased cortisol reaction upon psychosocial and physiological stress, with no difference found between the two types of stress (Petrowski, Wichmann, & Kirschbaum, 2018).

Many clinical conditions have been associated with altered HPA axis activity, largely relating to autoimmunity, inflammation, metabolism, and growth. Anorexia nervosa, obsessive-compulsive disorder (OCD), panic disorder, diabetes mellitus, childhood sexual abuse, hyperthyroidism, Cushing's syndrome, alcohol abuse, and pregnancy all have associations with increased or prolonged HPA axis activity. Adrenal insufficiency, seasonal depression, chronic fatigue syndrome, fibromyalgia, hypothyroidism, rheumatoid arthritis, and premenstrual tension syndrome all decrease HPA axis activity (Tsigos et al., 2000).

Cortisol

The two main classes of stress hormones are glucocorticoids, named cortisol in humans, and catecholamines, which are adrenaline and noradrenaline (Lupien et al., 2007).

Cortisol has its own 24-hour circadian rhythm, with the highest cortisol concentrations being in the early morning and slowly declining in the late afternoon, evening, and nocturnal period, with a sharp increase after the first few hours of sleep (Lupien et al., 2007; Tsigos et al., 2000). However, this is based on a traditional wake-sleep cycle, and studies have shown alterations based on age groups and altered wake times (Lupien et al., 2007). Older adults tend to perform better on tasks in the morning, while younger adults perform better in the afternoon (Hasher, Zacks, & Rahhal, 1999). The time of day at the time of testing could also be an important factor influencing the effects of stress-related elevations in glucocorticoids. In addition, high doses of endogenous and exogenous cortisol can cause cognitive impairments (Lupien et al., 2007). This circadian rhythm is controlled by the "CLOCK" system, which is made up of the suprachiasmatic nuclei of the hypothalamus and several extra-hypothalamic components. The system can be altered by changes in physical activity, feeding, and lighting, and disrupted in the event of a stressor (Tsigos et al., 2000).

Some natural differences have been found in individuals regarding their levels of cortisol. An upright position or a change from dim to brighter light can increase levels of cortisol (Vgontzas et al., 2004). Individual differences in self-esteem and locus of control, positive early-life attributes that can modify the appraisal of environmental stressors, are associated with related changes in HPA regulation in both young and elderly people (McEwen & Gianaros, 2010; Pruessner et al., 2005).

Several conditions are associated with altered cortisol sensitivity, including anxiety, posttraumatic stress disorder, asthma, rheumatoid arthritis, cardiovascular disease, inflammatory bowel disease, and autoimmune diseases (Slavich & Irwin, 2014). In addition, anticipation of tasks can alter levels of cortisol, with anticipation of

exhausting exercise showing comparable cortisol increases as completion of said exercise (Lupien et al., 2007).

Neuroimaging Studies of Stress

Psychological stress is associated with reduced activity in the medial orbitofrontal cortex and the anterior cingluate cortex (ACC), reduced activity in dorsolateral prefrontal cortex, and a distinct deactivation of a cluster of limbic system structures, including the hippocampus, hypothalamus and amygdala (Dedovic et al., 2009; Pruessner, Champagne, Meaney, & Dagher, 2004; Pruessner et al., 2008; Soliman et al., 2008; Wang et al., 2007, 2005). Functional neuroimaging studies have shown that the HPA axis is reliably engaged by stressors that involve completing demanding and uncontrollable cognitive challenges with added negative social evaluation (Dickerson & Kemeny, 2004; McEwen & Gianaros, 2010). Studies using serial subtraction with verbal feedback or computerized mental arithmetic task with a built-in social evaluation have shown increased cerebral blood flow in several brain regions, including the dorsolateral prefrontal cortex/ACC region, along with increases in the precuneus-superior parietal gyrus, insula, putamen, and inferior temporal region (Dedovic et al., 2009; Wang et al., 2005).

A modified version of the Trier Social Stress Test (TSST) administered during positron emission tomography (PET) scanning has shown significant associations between increased salivary cortisol levels and decreased activity in the hippocampus, amygdala, and hypothalamus (Kern et al., 2008; McEwen & Gianaros, 2010; Pruessner et al., 2008). *In vivo* imaging evidence in humans that reduced ACC volume is associated with HPA axis dysregulations, as indicated by a non-suppressed cortisol response to a dexamethasone challenge (MacLullich et al., 2006).

Cortisol changes elicited by the TSST administered outside of an MRI scanner were correlated with dorsal ACC activation during a social rejection task performed

inside of the scanner. Activation of the dorsal ACC, in addition to networked areas of the dorsal medial prefrontal cortex, were correlated with larger cortisol responses to TSST (Eisenberger, Taylor, Gable, Hilmert, & Lieberman, 2007).

Individuals who express lesser TSST-evoked cortisol reactivity also express lesser threat-related amygdala reactivity and greater regulatory activity in the ventral portion of the orbitofrontal prefrontal cortex (Taylor et al., 2010).

There is a relationship between chronic stress and changes in hippocampal morphology, even when the individuals are otherwise healthy (McEwen & Gianaros, 2010).

It is possible that pre-existing differences in brain morphology could increase vulnerability and decrease resiliency against life stress (Lupien et al., 2007; McEwen & Gianaros, 2010).

Stress, Affect, and Social Evaluation

While a stressful experience will typically cause a particular emotion, the same is not true of the reverse. Research into emotion and stress induce the two differently. Emotion is usually induced by a presentation of an emotional stimuli, such as words, images, or film, while stress is usually induced by having the individual involved in a usually stressful social situation, most commonly a public speaking task (Lupien et al., 2007).

While the presence of social evaluation can reliably induce a strong and significant activation of the HPA axis, individual response to psychological stressors is determined by situational and personality factors: half of a sample shows a significant stress response, while the other half will not (Dedovic et al., 2009; Dickerson & Kemeny, 2004; Pruessner et al., 2005, 2008).

Even acute stress can alter glucocorticoid sensitivity; research has shown that exposure to social stress induces glucocorticoid resistance in participants who were asked to give an impromptu speech in front of a socially rejecting panel of raters (Dickerson, Gable, Irwin, Aziz, & Kemeny, 2009; Rohleder, Schommer, Hellhammer, Engel, & Kirschbaum, 2001; Slavich & Irwin, 2014).

Interleukin-6

Immune System and Function

The human immune system coordinates the body's response to physical injuries and microbial infection in order to alleviate or prevent illness or harm. The first phase of the immune response, called innate immunity, is composed of immune and dendritic cells which circulate through the body in order to detect a variety of pathogens and initiate the inflammatory process in the event of injury or infection (Medzhitov, 2007; Slavich & Irwin, 2014). This response is nonspecific, does not provide continual protection, and lasts only minutes or hours. The second phase, called adaptive immunity, involves an increase and release of white blood cells to target the pathogen based on previous exposure to the pathogen, which can take days to fully develop (Barton, 2008; Slavich & Irwin, 2014). The inflammatory response in adaptive immunity can occur at the site of injury or infection, or throughout the body. Once the pathogen has been cleared, the response must be downregulated, in order to return the body to homeostasis (Slavich & Irwin, 2014).

In addition, the human immune system is split between the central and peripheral nervous system, and these two systems interact using pro-inflammatory and anti-inflammatory signaling proteins in order to maintain homeostasis (Morimoto & Alexopoulos, 2011; Young, Bruno, & Pomara, 2014). The peripheral immune system activates the innate immune system and activates the inflammatory process via the

release of cytokines (Young et al., 2014). In the central immune system, microglia release adenosine triphosphate, neurotransmitters, cytokines, ion changes, or loss of inhibitor molecules (Hanisch & Kettenmann, 2007; Morimoto & Alexopoulos, 2011) and activate astrocytes which produce additional cytokines, chemokines, and other molecules for the support of damaged neurons. Microglia additionally participate in tissue repair by removing cytokines and pathogens and by secreting wound-healing factors. Prolonged activation of microglia can lead to cell death via excessive release of quinolinic acid, which is a powerful excitotoxin (Dilger & Johnson, 2008; Morimoto & Alexopoulos, 2011).

Cytokines are polypeptides that primarily regulate the immune response to injuries, infections and other stressful events the organism is exposed to via removal of damaged cells, tissue repair, neuroregeneration, and clearing infections (Erta et al., 2012; Morimoto & Alexopoulos, 2011). They are responsible for the generation, stimulation, and differentiation of multiple cell types, as well as for the control of other cytokines that may potentiate or inhibit the synthesis of the biological effects or protein products of other cell types or proteins (Curfs, Meis, & Hoogkamp-Korstanje, 1997). When microglia and astrocytes begin producing pro-inflammatory cytokines, these cytokines bind to cytokine receptors in the CNS, which promotes the release of serotonin, dopamine, and norepinephrine (Slavich & Irwin, 2014). As cytokines are large proteins with hydrophilic properties, they do not cross the blood-brain barrier via passive transport efficiently; however, they can enter the central nervous system in areas where the blood-brain barrier is incomplete, contains saturable active transporters, or where there is a higher permeability (Slavich & Irwin, 2014; Young et al., 2014).

Cytokines also play a larger physiological role, including causing redness, heat, swelling, and pain at the site of infection, as well as the control of cells of the liver, heart,

and endocrine system (Erta et al., 2012; Slavich & Irwin, 2014). Cytokines include interleukins, tumor necrosis factors, lymphotoxins, interferons, colony-stimulating factors, chemokines, and miscellaneous cytokines (Curfs et al., 1997). Cytokines which upregulate inflammation are considered pro-inflammatory, while cytokines that downregulate inflammation are considered anti-inflammatory (Slavich & Irwin, 2014). Pro-inflammatory cytokines can also promote an increased vascular permeability in order to allow the immune cells to leave the blood vessels to target additional pathogens (Slavich & Irwin, 2014). When pro-inflammatory cytokines overpower anti-inflammatory cytokines, an exaggerated sickness response occurs (Dantzer, 2009), and if activation is prolonged, damage to the host can occur (Slavich & Irwin, 2014). TNF-α, IL-1 and IL-6 are considered the main pro-inflammatory cytokines, with IL-6 acting as the main circulating cytokine (Tsigos et al., 2000).

Interleukin-6 in the Body

Interleukin-6 (IL-6) is a cytokine which performs several tasks within the body, including controlling the change from innate immunity to acquired immunity (Erta et al., 2012). IL-6 binds to class I cytokine receptors and recruits glycoprotein 130 (gp130) for signal transduction (Boulanger, Chow, Brevnova, & Garcia, 2003; Erta et al., 2012). IL-6 is involved in many bodily functions in conjunction with immune system properties in humans and has roles in endocrine, autocrine, and paracrine signaling (Papanicolaou & Vgontzas, 2000). IL-6 interacts with several other cytokines: it inhibits tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1) (Dantzer, 2009; Papanicolaou, Tsigos, Oldfield, & Chrousos, 1996), and is potentiated by IL-1α and IL-1β (Young et al., 2014). Its production is suppressed by cortisol and estrogens, and stimulated by catecholamines (Papanicolaou & Vgontzas, 2000).

IL-6 activates chemokines, small polypeptides which redistribute immune system cells to sites of injury or infection, in order to survey the body to screen for pathogens and recruit other immune cells if successful (Slavich & Irwin, 2014). IL-6 also promotes the differentiation of T cells and B cells, which are lymphocytes that kill pathogens introduced to the body through physical wounding. Other cells over which IL-6 has control are hepatocytes (cells in the liver), hematopoietic progenitor cells (cells which produce blood cells and platelets), and neural stem cells (Erta et al., 2012). IL-6 also induces the production of C-reactive protein, which in combination with other cytokines can raise body temperature, heart rate, respiratory rate, and induce fever, which helps accelerate healing (Dantzer, 2009; Slavich & Irwin, 2014). In addition, they also promote social-behavioral withdrawal, which in humans allows the individual to recover and reduce the likelihood of infection spreading to others in the environment (Slavich & Irwin, 2014). At high enough levels, IL-6 can suppress testosterone production in men (Tsigos et al., 2000).

Interleukin-6 in Biofluid

IL-6 has separate effects based upon the biofluid tested. For example, elevated levels of IL-6 in serum have been strongly associated with depressed patients, while elevated levels of IL-6 in cerebrospinal fluid (CSF) have mixed results (Young et al., 2014).

Plasma IL-6 is secreted through 24-hour pulsations (Gaines et al., 2015). IL-6 in plasma has been shown to be affected by blood drawing technique; Haack, Kraus, Schuld, Dalal, Koethe & Pollmächer (2002) were able to demonstrate a significant increase in local IL-6 production when drawing via IV catheter as opposed to needle stick.

Clinical Conditions Related to Interleukin-6

Inflammatory mechanisms contribute to the risk of a wide spectrum of medical conditions; increases of markers of inflammation, including IL-6, can predict or even facilitate such conditions (Irwin, Olmstead, & Carroll, 2016). Following weight gain, IL-6 levels are increased, and obese patients typically exhibit increased circulating levels of IL-6 (Kyrou & Tsigos, 2009). IL-6 has been related to multiple sclerosis and Alzheimer's disease and has been shown to be protective against excitotoxicity related to Huntington's disease and Parkinson's disease (Erta et al., 2012). IL-6 expression was found by Wei et al. (2012) to be significantly increased in the cerebellum tissue of subjects who had autism in comparison to age-matched controls. Gaines et al. (2015) analyzed sleep apnea severity and levels of plasma IL-6 and found no differences between the severities.

Straub et al. (2000) compared postmenopausal women and found that women with hormone replacement therapy had significantly lower levels of IL-6 in serum as opposed to women not on hormone replacement therapy, even with controlling for age, antihypertensive therapy, smoking, and blood pressure. Another study looking at older women showed that chronic stressors are associated with significant elevations in IL-6 outside of elevations associated with normal aging. (Lutgendorf et al., 1999).

In a comparison of patients with panic disorder (PD) diagnosis and remitted PD patients, Belem da Silva et al. (2016) found significantly higher mean levels of serum IL-6 in current PD patients. Panic severity was additionally related to levels of IL-6, with higher panic severity being related to higher IL-6 concentrations. In PD patients, more severely affected patients demonstrate a greater peak level of IL-6, as demonstrated in Petrowski et al. (2018). A higher IL-6 concentration has also been found in schizophrenic patients as compared to healthy controls (Erta et al., 2012).

IL-6 has been particularly researched in terms of its relationship with depression. "Sickness behavior," the psychological and behavioral responses of an individual fighting infection, includes subjective feelings of sickness such as malaise, fatigue, reduced appetite, and muscle and joint aches. Sickness behavior is triggered by pro-inflammatory cytokines, including IL-6, and as this behavior shares many symptoms with major depression, it has led to a belief that depression may be a behavioral response to pro-inflammatory changes (Dantzer, 2009; Morimoto & Alexopoulos, 2011).

Research into specifically IL-6 concentrations and major depressive disorder usually involve the comparison of concentrations of IL-6 in depressed patients as compared to healthy controls; studies have shown a higher concentration of plasma, peripheral blood, and serum IL-6 in depressed patients, even when controlling for other inflammatory variables (Dantzer, 2009; Erta et al., 2012; Morimoto & Alexopoulos, 2011; Munzer et al., 2013; Slavich & Irwin, 2014). Studies into IL-6 concentrations in CSF have not been as clear, with some researchers believing this may be due to IL-6's pro- and anti-inflammatory properties (Young et al., 2014). Higher concentrations of IL-6 have also been associated with an increased suicide risk, and Lindqvist et al. (2009) found a relationship between elevated IL-6 and a higher likelihood of a violent suicide attempt (Morimoto & Alexopoulos, 2011; Young et al., 2014). Elevations of IL-6 have also been found to predict development of depression symptoms (Slavich & Irwin, 2014).

Patients with SSRI-resistant depression have been shown to have higher plasma IL-6 concentrations than healthy controls or even formerly SSRI-resistant patients (Morimoto & Alexopoulos, 2011; O'Brien, Scully, Fitzgerald, Scott, & Dinan, 2007; Young et al., 2014). Studies investigating the link between IL-6 and antidepressants have found that select antidepressants may reduce serum cytokines (Morimoto & Alexopoulos, 2011; Munzer et al., 2013) at a level that matches the reduction of symptoms (Slavich &

Irwin, 2014), and high IL-6 levels were associated with a poorer response to antidepressant treatment (Munzer et al., 2013).

IL-6 appears to have an important role in the pathophysiology of depression. Munzer et al. (2013) investigated several antidepressants and their effect on IL-6 on whole blood *in vitro* and found that of citalopram, escitalopram, and mirtazapine, citalopram actually increased IL-6 levels, while there were no significant differences in IL-6 levels for the other two antidepressants in comparison to controls. Another *in vitro* study analyzed lymphocytes of depressed patients and found significantly stronger activation of IL-6 production than in lymphocytes of healthy controls (Heiser, Lanquillon, Krieg, & Vedder, 2008; Munzer et al., 2013).

Manipulation of Interleukin-6

Studies involving a change in IL-6 levels tend to either be as part of a natural inflammatory period or as part of a physiological stressor. Individuals caring for the elderly had a prolonged elevation of IL-6 following administration of the influenza vaccine in comparison to age-matched controls, lasting up to four weeks post-vaccine (Morimoto & Alexopoulos, 2011). For acute stress, the TSST results in a significant increase of IL-6 in both depressed patients and healthy controls (Steptoe, Hamer, & Chida, 2007).

Neuroimaging and Interleukin-6

Neuroimaging studies investigating the effect of IL-6 on the brain have found levels of IL-6 covaried inversely with hippocampal gray matter volume (McEwen & Gianaros, 2010). Increases in IL-6 are associated with greater neural activity in the anterior insula and dorsal ACC, and endotoxin-induced increases in IL-6 were also related to greater activity in several neural regions including the medial and dorsomedial prefrontal cortex, posterior cingulate cortex, and precuneus (Slavich & Irwin, 2014).

FMRI studies have shown increased serum pro-inflammatory cytokine concentrations correlated with increased activity of the subgenual ACC (Harrison et al., 2009), dorsal ACC, and anterior insula (Slavich, Way, Eisenberger, & Taylor, 2010; Young et al., 2014).

Sleep and Stress

Research into the interaction between sleep and stress has found an association with high stress and low sleep quality. Stress can cause many types of sleep difficulty, including restless sleep, waking up too early, later rise times, and mid-sleep awakenings (Almojali et al., 2017; Amaral et al., 2018). Sleep difficulties are also associated with perceived stress, catastrophizing, and negative affect (Amaral et al., 2018).

Studies have looked into specific groups and their response to sleep and stress, including in medical students and individuals with chronic insomnia. Almojali et al. (2017) showed that in a sample of medical students, a high level of stress was a major predictor and contributor to poor sleep quality, with a 22% increase in prevalence of poor sleep quality from non-stressed students to stressed students. Vgontzas et al. (1998) found that in chronic insomnia, the level of activity in the HPA axis is directly proportional to the degree of sleep disturbance.

A Germain, Buysse, Ombao, Kupfer, and Hall (2003) study found that when subjects were told they would have to give an evaluated speech in the morning, the subjects had a decrease of late-night REM and an increase in REM-density across REM periods; after the speech, a slower rate of increase across successive REM periods was found. A reducted REM-sleep latency has been found to be an objective indicator of depression and suicide risk (Vandekerckhove & Cluydts, 2010).

Cortisol has been found to decrease after 24 hours of total sleep deprivation (Sauvet et al., 2017); however, sleep restriction is not associated with a change in 24-hour cortisol levels (Pejovic et al., 2013).

Studies investigating naps have found that cortisol decreases during a midday nap lasting either 30 minutes or 2 hours (Faraut et al., 2015). Vgontzas et al. (2007) found that daytime napping following a night of total sleep loss significantly lowered levels of cortisol during the nap, and significanty increased cortisol post-nap in comparison to non-nappers. In addition, the nap significantly improved levels of objective and subjective sleepiness. These studies suggest that relatively lower levels of cortisol are associated with sleep, while higher levels are associated with a waking state. However, following a week of mild sleep restriction, no such change in cortisol is found, which may indicate that a more stressful sleep deprivation is required in order for napping to induce an antistress effect (Pejovic et al., 2013; Vgontzas et al., 2007, 2004). Sleep restriction does impact the circadian secretion of cortisol; peak cortisol secretion was shifted 2 hours earlier than baseline after sleep restriction, with no difference in the nadir time (Vgontzas et al., 2004).

These results are likely due to the biological relationship between sleep and stress: activation of the stress system (specifically the HPA axis) leads to arousal and sleeplessness, while sleep has an inhibitory influence on the stress system (Almojali et al., 2017; Vgontzas et al., 1998). Both CRH and ACTH induce increased waking, and an increase of endogenous level of cortisol is associated with increased arousal and sleep disruption (Vgontzas et al., 2007).

Interleukin-6 and Stress

A negative feedback loop exists between endogenous cortisol and plasma IL-6 (Papanicolaou et al., 1996). IL-6 plays a major role in the immune stimulation of the

HPA axis and has itself been shown to be a potent direct activator of the HPA axis (Erta et al., 2012; Papanicolaou et al., 1996; Tsigos & Chrousos, 2002; Tsigos et al., 2000). IL-6 also has the capability of modifying the sensitivity of glucocorticoid receptors (Munzer et al., 2013). In turn, cortisol inhibits the immune response, including decreasing production of pro-inflammatory cytokines; a reduction in cortisol secretion usually suggests increased pro-inflammatory cytokine reactions, including IL-6 (Papanicolaou et al., 1996; Petrowski et al., 2018; Tsigos & Chrousos, 2002).

Psychological stress, including social evaluation, conflict, or rejection, can trigger significant increases in inflammatory activity (Slavich & Irwin, 2014), and both pro- and anti-inflammatory cytokines have been shown to increase upon psychosocial stress (Petrowski et al., 2018; Steptoe et al., 2007), especially IL-6 (Slavich & Irwin, 2014). Neural systems involved in processing physical pain also have a role in processing social pain; this response may indicate that psychological stressors up-regulate systemic inflammation, which can, in turn, promote greater neural sensitivity to psychological stress. Over time, this could lead to a positive feedback loop that increases overall levels of inflammation and lead the individual to be at high risk for stress-related conditions (Slavich & Irwin, 2014).

Studies comparing levels of stress and IL-6 have shown an association with higher levels of circulating IL-6 and individuals with more stress. Slopen et al. (2013) conducted a longitudinal study where exposure to acute life stress was assessed at seven time points between 1.5 and 8 years old. When levels of plasma IL-6 were measured at age 10, greater cumulative stress exposure before age 8 predicted higher levels of IL-6. Increased levels of IL-6 have been related to lower socioeconomic status in children (Appleton et al., 2012; Carroll, Cohen, & Marsland, 2011; Slavich & Irwin, 2014; Taylor, Lehman, Kiefe, & Seeman, 2006), adolescents (Miller & Cole, 2012; Slavich & Irwin,

2014) adults (Gruenewald, Cohen, Matthews, Tracy, & Seeman, 2009; Petersen et al., 2008; Pollitt et al., 2007; Slavich & Irwin, 2014), and older adults (Carpenter, Gawuga, Tyrka, & Price, 2012; Carroll et al., 2013; Gouin, Glaser, Malarkey, Beversdorf, & Kiecolt-Glaser, 2012; Kiecolt-Glaser et al., 2011). Exposure to a childhood environment characterized by unpredictability and interpersonal stress has been related to greater *in vitro* LPS-stimulated IL-6 production in adolescents (Miller & Chen, 2010).

One study involving manipulated psychological stress and IL-6 took married couples and asked them to engage in a social support interaction and a hostile interaction. Married couples evaluated as having high hostility were found to have a significantly higher increase of IL-6 following the hostile interaction than following the social support interaction, while couples evaluated as having low hostillity saw no change in either case (Kiecolt-Glaser et al., 2005; Slavich & Irwin, 2014).

Studies have also combined manipulated stress and stress history. Carpenter et al. (2010) found that individuals with higher early-life stress exhibited greater IL-6 responses to the TSST than individuals with little or no early-life stress, even though at baseline these levels were not significantly different. Slavich, Way, Eisenberger and Taylor (2010) found that healthy young adults who were more sensitive to social rejection exhibited greater IL-6 responses to the TSST. Jaremka et al. (2013) found that participants with higher feelings of trait loneliness exhibited greater production of IL-6 in response to the TSST than those who had lower feelings of loneliness.

Sleep and Interleukin-6

Research suggests that IL-6 is a sleepiness-mediating cytokine. Daytime levels of IL-6 increase after total (Sauvet et al., 2017; Shearer et al., 2001; Vgontzas et al., 2004) or partial (Vgontzas et al., 2007, 2004) sleep loss. Even a restriction of 2 hours a night for 7 days is associated with significant increases of IL-6 in healthy adults (Vgontzas et al.,

2004). Sleep disruption is also associated with an increase in plasma levels of IL-6 (McEwen & Gianaros, 2010). However, IL-6 levels are higher in total sleep deprivation than in partial sleep deprivation (Shearer et al., 2001; Vgontzas et al., 2007). Mean daytime levels of IL-6 post-deprivation are significantly higher than pre-deprivation, and negatively related to the amount of nocturnal sleep (Vgontzas et al., 1999).

Daytime napping following a night of total sleep deprivation significantly decreased IL-6 levels during the nap and IL-6 tended to remain lower in the post-nap period as compared to the no-nap group (Faraut et al., 2015; Vgontzas et al., 2007). Daytime naps for no longer than 30 minutes after a night with only 2 hours of sleep restores salivary IL-6 levels to baseline levels (Faraut et al., 2015). Recovery sleep also has an impact on IL-6 levels. Two days of extended recovery sleep can reverse the IL-6 increase caused by short-term sleep loss (Pejovic et al., 2013).

IL-6 is usually secreted in a biphasic circadian pattern with the first zenith at 5:00AM, and the first nadir at 8:00AM; the second phase hits its zenith at 7:00PM, with its nadir two hours later. During sleep deprivation, daytime oversecretion and nighttime undersecretion occurs (Vgontzas et al., 1999), with the strongest differences between 4:30AM and 6:30 AM.

Elevated IL-6 is also implicated in disorders dealing with excessive sleepiness, including sleep apnea and narcolepsy, and may suggest that IL-6 plays a major role in sleepiness and fatigue (Gaines et al., 2015; Vgontzas et al., 2007).

Interleukin-6, Stress, and Sleep Deprivation

Some research has looked into IL-6, sleep deprivation, and stress, although these studies largely only compare cortisol and IL-6 levels. The interaction of cortisol and IL-6 can affect the level of sleepiness and fatigue, with relatively higher IL-6 and relatively low cortisol being associated with sleepiness, and relatively higher IL-6 and high cortisol

being associated with poor sleep and fatigue (Vgontzas et al., 2007). Sleep deprivation has been found to result in decreased cortisol secretion and an elevation of circulating IL-6 (Vgontzas et al., 1999, 2004), and recovery sleep reverses the increase of IL-6 and decrease of cortisol (Pejovic et al., 2013; Vgontzas et al., 2007). However, some studies have shown a change in IL-6 and not in cortisol (Faraut et al., 2015), suggesting that IL-6's anti-inflammatory properties may come into play during sleep deprivation, and should be studied further.

CHAPTER III:

METHODS

Participants

Eleven adults aged 18-45 (M = 26.27, SD = 6.60) participated in this study, with no preference towards gender, race, or ethnicity. Participants were recruited from the UHCL online participant pool, via flyers, and through referrals. Health status was evaluated before inclusion in the experiment and participants were screened to ensure those chosen are in good general health and free from psychological or psychiatric illness and sleep disorders; additionally, participants that had more than a 30-minute commute to UHCL main campus were excluded for safety reasons. A majority of participants were female (63.63%, 7/11) and Hispanic (45.45%, 5/11). One participant of the eleven was removed due to noncompliance and scores on both the Social Interaction Anxiety Scale and Pittsburgh Sleep Quality Index that were two standard deviations above the mean.

Experimental Design

Participants were randomly assigned to one of two groups: the sleep deprivation group or the control group.

Sleep Deprivation Group

The sleep deprivation group arrived at the laboratory two hours before their normal bedtime. Participants were asked to refrain from taking naps and eating or drinking anything containing caffeine, such as coffee or chocolate, the day before and during testing. Participants were monitored continuously by the research staff to ensure wakefulness. The subjects were additionally monitored with an activity tracker (Fitbit, Inc., San Francisco) attached to the wrist of the nondominant hand throughout the experiment to assess subject compliance. Throughout sampling, the subjects were ambulatory, and non-stimulating activities such as reading or playing board games were

made available to assist with wakefulness. To self-report measures of chronic stress, participants completed the Perceived Stress Scale (PSS), and to self-report sleep quality, participants completed the Pittsburgh Sleep Quality Index (PSQI). Participants also completed the Karolinska Sleepiness Scale (KSS) in order to self-report their current level of sleepiness, and completed the Social Interaction Anxiety Scale (SIAS) to compare with their eventual IL-6 levels after a psychosocial stressor. The sleep deprivation group stayed awake for 8 additional hours after completing the evening tasks. The acute psychosocial stressor, in this case the Trier Social Stress Test (TSST), began 8 hours after the participants' normal bedtime. Saliva samples were collected at one time point before the TSST, one point immediately after, and one final point after ten minutes, giving a total of three saliva samples. When saliva was collected, participants completed the Positive and Negative Affect Schedule.

Control Group

For the control group, the procedures were the same as for those in the sleep deprivation group, but participants were allowed to return home following baseline testing and asked to go to sleep immediately. Participants were asked to report back to the laboratory in the morning for additional testing, and were administered the same battery of tasks administered to the sleep deprivation group. To assess at-home compliance, participants wore a FitBit through the duration of the study.

Measures

Perceived Stress Scale

The Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983) is a 14-item scale developed in accordance with the transactional perspective. The PSS provides a global appraisal of stress by asking participants to report whether their lives seem to be unpredictable. The PSS is sensitive to several different types of stressors,

including non-occurrence of events, ongoing life circumstances, events occurring in the lives of friends and relatives, and expectations concerning future events (Cohen, Williamson, Spacapan, & Oskamp, 1988; Ezzati et al., 2014).

Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) is a 19-item questionnaire developed to provide a standardized measure to discriminate between 'good' and 'poor' sleepers by asking questions related to seven quantitative and subjective aspects of sleep quality. The PSQI generates seven equally weighted component scores, detailing subjective sleep quality, sleep latency, sleep duration, habitual sleep efficacy, sleep disturbances, use of sleep medications, and daytime dysfunction. These scores assess sleep quality and potential sleep disturbances over a one-month period. The seven scores are totaled together, giving a global score of a range of 0 (indicating no difficulty) through 21 (indicating severe difficulty), with a general cutoff for good sleep quality at 5 points or less.

Karolinska Sleepiness Scale

As the amount of sleep individuals need varies, how sleepy a test participant believes themselves to be is an important variable to consider. A popular test used to determine subjective sleepiness is the Karolinska Sleepiness Scale (KSS), which measures subjective sleepiness by asking the individual to rate themselves on their feeling of drowsiness and fatigue (Drummond et al., 2000; Shekleton et al., 2014).

Social Interaction Anxiety Scale

The Social Interaction Anxiety Scale (SIAS) (Mattick & Clarke, 1998) was developed to assess the fear of general social interaction, corresponding to the generalized type of social phobia as described in the DSM-III-R. Social interaction anxiety for the questionnaire is defined as distress when meeting and talking with other

people. The SIAS contains 20 items in a Likert scale ranging from 0 (not at all characteristic or true of me) to 4 (extremely characteristic or true of me). The SIAS is scored by summing the ratings after reverse scoring 2 items, with a total ranging from 0 to 80, with higher scores representing higher levels of social interaction anxiety.

Trier Social Stress Test

Participants were asked to perform the Trier Social Stress Test (TSST), a simulated public speaking task (Birkett, 2011). Initially, each participant rested in a darkened, quiet room for 30 minutes. The participants were then read a scenario in which they had been accused of shoplifting and were asked to prepare a speech defending themselves. The participant was informed that their speech performance would be judged by an expert panel for content, quality, and duration. The participant was also asked to perform a mental arithmetic task which they were told would give those who review the performance additional information about their believability and convincingness (Kirschbaum, Pirke, & Hellhammer, 1993). The participants were given 3 minutes to prepare and 5 minutes to deliver their speech to a panel of three individuals. Following the speech, the participant then performed the mental arithmetic task for an additional 5 minutes.

Positive and Negative Affect Schedule

Positive affect is defined as the amount an individual feels enthusiastic, alert, and active, with a low positive affect being characterized by sadness and lethargy as well as an association with depression (D. Watson, Clark, & Tellegen, 1988). In contrast, negative affect details distress and mood states including anger, disgust, and fear, with high negative affect being associated with anxiety, stress, and neuroticism. The Positive and Negative Affect Schedule (PANAS) uses a Likert scale ranging from 1 (very slightly or not at all) through 5 (extremely), answering to what extent a person feels between 20

items (10 associated with positive affect, and 10 with negative affect) at a given time. The PANAS has been used for the present moment, a specific time period (such as the last week), or in general. The PANAS is scored by summing the items for the positive affect scale and the negative affect scale separately.

Physiological Measures of Stress

Saliva samples were collected from each participant during the initial baseline visit and at one time point before the Stress Test and one time point afterward, for a total of three saliva samples. The sample tubes were stored in a -20°C freezer. IL-6 levels were quantified via human enzyme immunoassay (EIA) kit per the manufacturer's instructions (Salimetrics LLC, USA). Samples were brought to room temperature and centrifuged at 1500 g for 15 minutes, with the clear top-phase of the sample pipetted into appropriate test tubes and assayed normalized to total protein concentration. Absorbance readings were performed on a spectrophotometer using 450 nm as the primary wavelength. Magellan software was used for data reduction to calculate concentration through a 4-parameter regression curve (Tecan Trading AG, Switzerland). The lower detection limits for IL-6 were 0.07 pg/ml.

Data Analysis

A mixed ANOVA (sleep condition x stress condition) inferential statistical procedure was used to compare IL-6 levels of the sleep-deprived and control subjects before the TSST, immediately after the TSST, and 10 minutes after the TSST. A second mixed ANOVA was used to compare PANAS scores in order to analyze the participants' perceived stress. T-tests were conducted to ensure the groups were not significantly different for any specific feature stress or sleep-related feature ($p < 0.05$, no multiple comparisons corrections). All statistical analyses were performed in SPSS (SPSS, INC., Chicago, Illinois).

CHAPTER IV:

RESULTS

No significant differences were found between the two groups in terms of their KSS ($t(8) = 1.132$, $p = .290$), PSS ($t(8) = 0.746$, $p = .477$), SIAS ($t(8) = 1.031$, $p = .333$), or PSQI scores ($t(8) = 0.079$, $p = .939$).

Two two-factor mixed ANOVAs were conducted to determine a statistically significant difference between sleep condition and stress condition on PANAS scores, both for negative affect and positive affect. For negative affect, Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(2) = 13.732$, p = .0011. There was no significant effect of stress condition on negative affect, $F(1.076, 8.605) = 3.249$, $p = .105$, $\eta^2 = .289$, once correcting for sphericity, and no significant effect of sleep condition on negative affect, $F(1,8) = 1.509$, $p = .333$, $\eta^2 = .117$ (see Figure 1). For positive affect, Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(2) = 3.308$, p = .191. There was no significant effect of stress condition on positive affect, $F(2,16) = 0.042$, $p = .959$, $\eta^2 = .005$, and no significant effect of sleep condition on positive affect, $F(1,8) = 0.462$, $p = .516$, $\eta^2 = .055$ (see Figure 2).

A two-factor mixed ANOVA was conducted to determine a statistically significant difference between sleep condition and stress condition on IL-6 levels. Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated, $\chi^2(2) = 7.231$, $p = .027$. There was no significant effect of stress condition on IL-6 levels, $F(1.217,9.732) = 0.403$, $p = .562$, $\eta^2 = .052$, once correcting for sphericity. There was no significant effect of sleep condition on IL-6 levels, $F(1,8) = 0.032$, $p = .862$, $\eta^2 = .004$. There was also no significant stress condition and sleep condition

interaction, $F(1.217,9.732) = 0.385$, $p = .590$, $\eta^2 = .046$, once correcting for sphericity (see Figure 3).

CHAPTER V:
DISCUSSION

Use of Interleukin-6 as a Marker of Sleep Deprivation and Stress

No statistically significant results were found in the course of this study, and we were unable to replicate any differences in IL-6 due to sleep deprivation, stress, or due to an interaction between the two. Much of this may be due to our sample size, which limits the validity and reliability of our findings. Without a significant result in any direction, it is difficult to claim that IL-6 levels in our participants were impacted in any meaningful way, even in directions that have been shown in multiple other studies researching IL-6.

The choice of measurement technique is important when assessing biomarkers that are potentially sensitive to the sleep debt status of the subject. Our use of a noninvasive measurement method by sampling saliva is a benefit due to having no methodological confounders, unlike intravenous (IV) catheters, which have been found to increase local IL-6 production (Haack et al., 2002; Irwin, Carrillo, & Olmstead, 2010) when used for repetitive blood sampling.

This study is also unique in its attempt to combine both sleep deprivation and social stress with their impact on IL-6, as opposed to separating the two. Given that sleep deprivation and stress are so closely linked, it is key to not exclude one in favor of the other when analyzing the effect on the body.

Study Limitations

While our study was able to control for many factors associated with sleep, stress, and IL-6 production, there are also known factors that affect sleepiness and alertness that we were not able to account for, including chronotype, distractions by environmental stimuli and noise (Landström, Englund, Nordström, & Åström, 1998), or boredom and motivational factors (Hull, Wright, & Czeisler, 2003; Mavjee & Horne, 1994; Minors &

Waterhouse, 1983) by the participants (Goel et al., 2013). In addition, since the control group went to their own home to sleep, we cannot be certain of factors including ambient temperature (Mavjee & Horne, 1994) and lighting conditions (Cajochen, Zeitzer, Czeisler, & Dijk, 2000; Frenda & Fenn, 2016; Goel et al., 2013; Phipps-Nelson, Redman, Dijk, & Rajaratnam, 2003). We also cannot rule out the possibility that our results might be due to inadequate sensitivity of the assay.

In addition, since about half of a sampled population will usually not produce a significant stress response to social evaluation (Dedovic et al., 2009; Dickerson & Kemeny, 2004; Pruessner et al., 2005, 2008), it may very well be possible that our sample has these effects as well.

Suggestions for Future Research

This study is best seen as a pilot study and should be continued on a larger scale in order to see what results may be possible. In addition, these effects need to also be investigated in the specific relevant populations including shift workers, first responders and military personnel.

Functional neuroimaging studies may identify abnormalities in activation and function of the brain associated with inflammation, and longitudinal studies could investigate whether the changes seen in function related to IL-6 and inflammation are predictive of future mental illness. Inflammatory profiles in conjunction with neuroimaging could also allow for more specific diagnosing of disorders and specific treatment plans.

Another avenue to be explored would be differences in results due to age, as participant age has been found to have an effect on both sleep need and cortisol levels (Vgontzas et al., 1998), as well as an effect on reaction to the research environment (Lupien et al., 2007).

Research seeks to explain the connections between sleep, stress, and immune system functioning, and this study acts as a starting off point for future research into IL-6 and other biological markers.

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APPENDIX A:

FIGURES

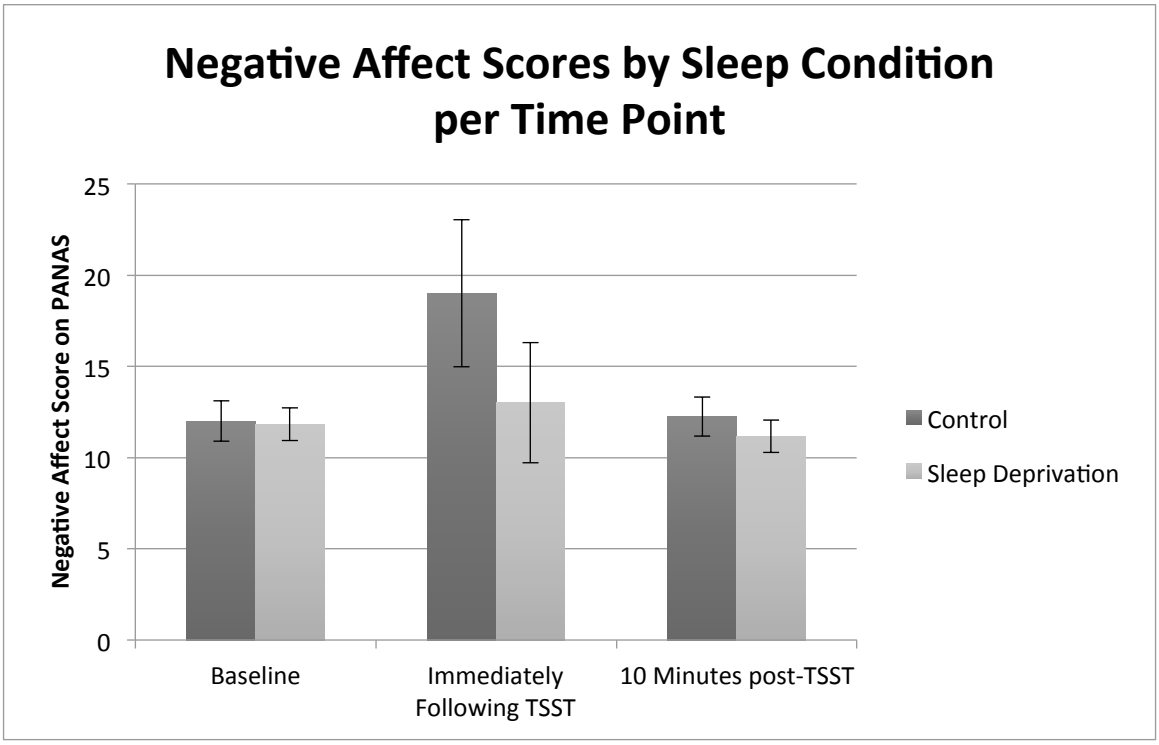

Figure 1

Negative Affect Scores by Sleep Condition per Time Point

Average scores on the negative affect section of the PANAS, as grouped by sleep condition and time point. Standard errors are represented in the figure by the error bars attached to each column. PANAS: Positive and Negative Affect Schedule; TSST: Trier Social Stress Test

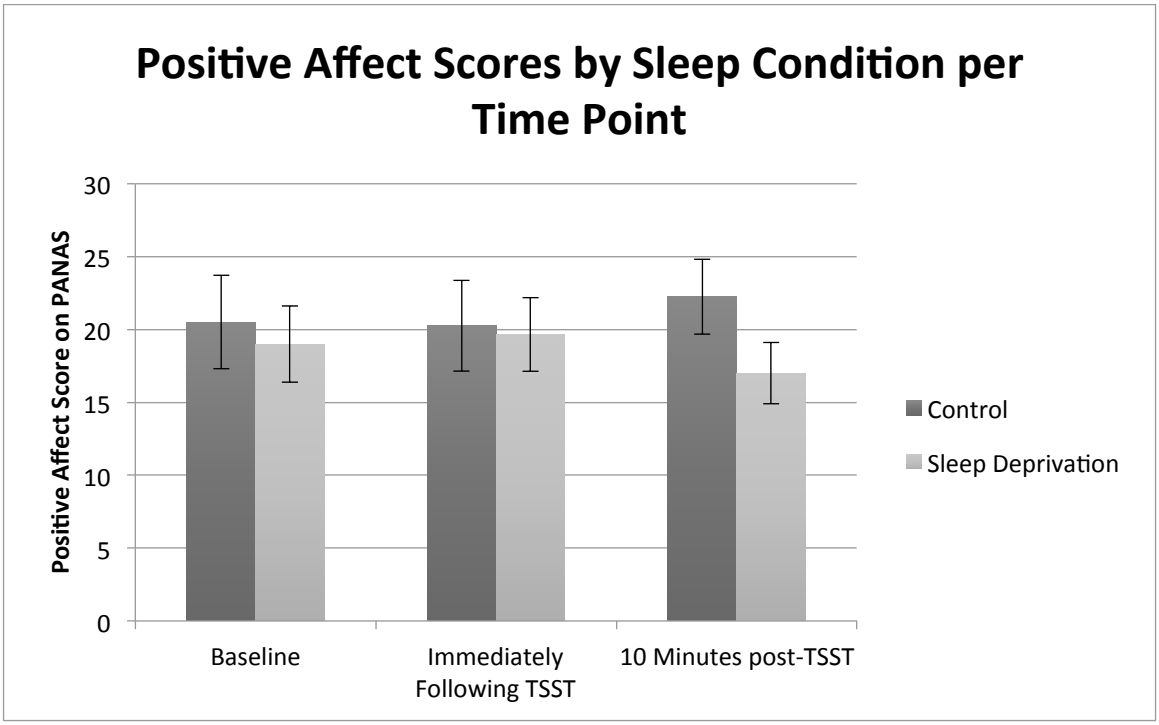

Figure 2

Positive Affect Scores by Sleep Condition per Time Point.

Average scores on the positive affect section of the PANAS, as grouped by sleep condition and time point. Standard errors are represented in the figure by the error bars attached to each column. PANAS: Positive and Negative Affect Schedule; TSST: Trier Social Stress Test

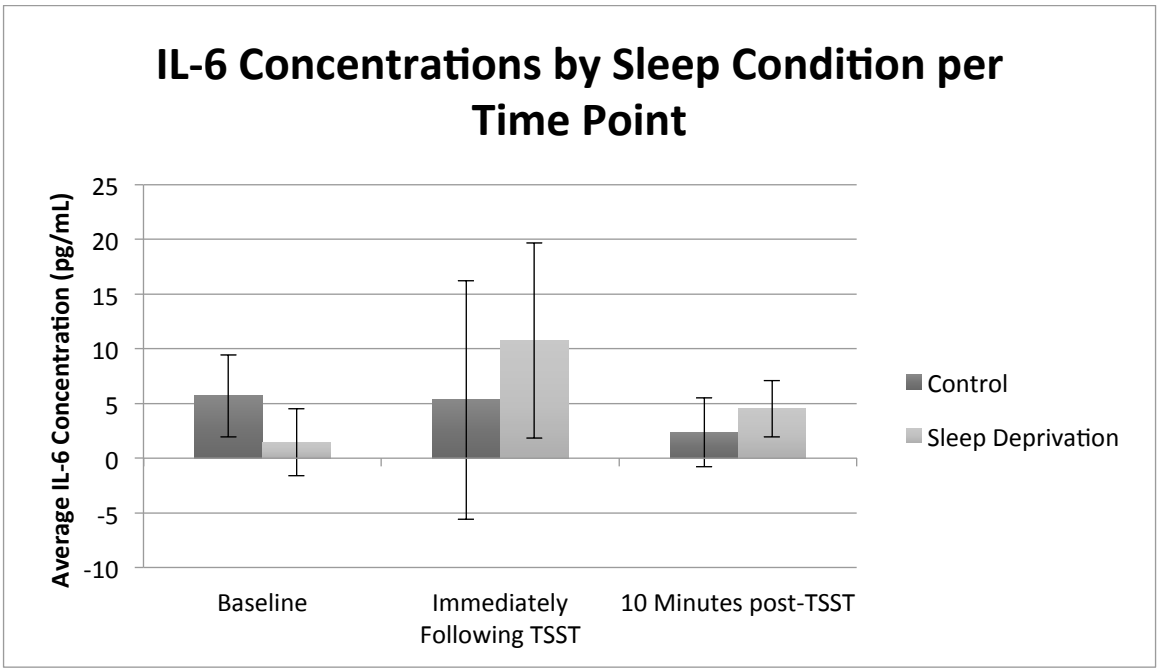

Figure 3

IL-6 Concentrations by Sleep Condition per Time Point

Average IL-6 concentration (in pg/mL) as grouped by sleep condition and time point. Standard errors are represented in the figure by the error bars attached to each column. TSST: Trier Social Stress Test; pg/mL: picograms per milliliter.