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Sex Differences in the Transitional Period between Slow-Wave Sleep and REM Sleep: A Novel Metric for Sleep Quality and Neuropsychiatric Disorders

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SEX DIFFERENCES IN THE TRANSITIONAL PERIOD BETWEEN SLOW-WAVE SLEEP AND REM SLEEP: A NOVEL METRIC FOR SLEEP QUALITY AND NEUROPSYCHIATRIC DISORDERS

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ABSTRACT

Sleep is an important biological process, and it is well-documented that sleep disturbances often precede neuropsychiatric disorders. Sleep has traditionally been divided into two basic stages, non-REM and REM sleep. These two stages are often considered to have distinct functions and are usually studied independently. However, an additional sleep stage, the transitional period between non-REM and REM sleep, has been described but not well investigated. While sex differences in the other stages of sleep have been documented and are deemed clinically relevant, no study has investigated sex differences in this transitional period. In the present study, this period is termed transition sleep is directly compared between male and female rats for the first time. Eight male and eight female adult rats were fitted with sleep-recording implants, and their sleep-wake activity was recorded every four days for two weeks. The results indicate that female rats spend more time in transition sleep and have a higher frequency of transition sleep episodes. Additionally, regression models of the percentage of time spent in each sleep stage revealed that the different sleep stages had larger effects on one another in the female rats compared to the males. This indicates that, for the females, the different sleep stages were more interdependent, pointing to a difference in the biological mechanisms that are involved in regulating transitions between sleep stages. These findings provide novel insight into sex differences in sleep and a new approach for investigating the link between sleep disturbances and psychiatric illness.

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CHAPTER ONE

INTRODUCTION AND GENERAL INFORMATION

What is Sleep?

A common misconception is that sleep is a passive state of unconsciousness[1]. This misconception can be easily dispelled by comparing sleep to anesthesia. One is, in fact, a complete loss of consciousness: You can't awaken from anesthesia until the anesthetic agent is removed from the system. Anesthesia prevents sensations, mental experiences, and memory formation. Under anesthesia, the brain is in a very passive, inactive state. While asleep, however, none of those things are true. We retain some amount of awareness of our surroundings, allowing us to be awoken if something surprising or potentially threatening happens. We have thoughts and dreams, which incorporate sensations and mental processes. We can remember our dreams, indicating that our memory systems have not been completely inactivated.

All of this illustrates what the past fifty years of sleep research has shown: sleep is an active process performed by the brain. The activity of the brain during sleep is much different than that of the brain during wakefulness, but there is definitely activity. Additionally, almost every animal with a central nervous system sleeps, and similar patterns of brain activity are exhibited by the sleeping brain across species [2]. This marks sleep as one of the most evolutionarily conserved behaviors we know to date, suggesting that brains cannot exist without it.

Brain Activity During Sleep

Brain activity can be measured in a wide variety of ways. For the purposes of sleep studies, local field potentials are used most often. Local field potentials are measured by microelectrodes implanted in neural tissue, which effectively measure the electrical activity of nearby neurons. The sum of action potentials and graded potentials around the membranes of the neurons produces an amount of voltage (μV) that can be measured by the electrode. This measured electrical activity is termed *electroencephalogram,* or EEG. Higher voltages in the EEG indicate more neuronal activity.

The EEG signals are processed in three major ways: the sampling rate, the high-pass filter and the low-pass filter. The sampling rate indicates how often measurements from the electrode are collected and are expressed in Hz (number of samples/second). The high- and low-pass filters, collectively referred to as the band-pass filter, determine the lowest and highest frequencies allowed in the sample, respectively. This filters out noise, producing cleaner signals.

EEG signals contain waves that show the rate at which certain voltages appear. For example, a 100 μV measurement might occur at a frequency of 12/sec (Hz), a 200 μV measurement might occur at a frequency of 40 Hz, and so on. These signals thus contain *frequency* (Hz) and *power* (μV) components. If the signal contains a certain frequency at a very high power, it indicates that

neurons around the microelectrode are firing at that frequency a high percentage of the time, illustrating the "rhythm" of the firing.

Signal frequencies are conventionally divided into 5 functional wavelengths: *delta* (0.5 – 3 Hz), *theta* (4 – 8 Hz) , *alpha* (9 – 12 Hz), *beta* (13 – 30 Hz), and *gamma* (> 30 Hz). Each frequency is believed to be involved in distinct processes, which are outside the scope of this study [3-11]. Generally, especially in signals taken from the cortex, higher frequencies indicate higher states of arousal. *Beta* waves dominate during periods of alert wakefulness, with *gamma* activity indicating high alertness and *alpha* activity indicating relaxation.

The Stages of Sleep

Sleep is not a uniform state. Human sleep has been broken down into as many as five different stages, but there are ultimately two distinct categories: Rapid Eye Movement (REM) sleep, and non-REM sleep. Rapid eye movements are the namesake of REM sleep and appear only in that state of consciousness. Otherwise, the two categories are separated by their similarity to waking brain activity. During REM sleep, the cortex is extremely active, as it is during wakefulness. During non-REM sleep, the cortex shows relatively low levels of activity. The pattern of alternations between wakefulness, non-REM and REM sleep over a specified period is termed *sleep architecture*.

Non-REM Sleep

The transition from wakefulness to non-REM sleep is well characterized and will only be briefly discussed here [12, 13]. In general, sleep onset is determined from a combination of circadian and homeostatic processes. Circadian processes influence daily rhythms of activity and are regulated by activity within the suprachiasmatic nucleus. Circadian processes help set the time frame for when sleep is most likely to occur. Homeostatic processes, on the other hand, indicate how much sleep is actually needed. The neuroanatomical mechanisms of sleep homeostasis are most centralized in the hypothalamic preoptic nucleus and thalamic reticular nucleus. Adenosine, a common metabolite of ATP, builds up with increased neuronal activity and plays a large role in indicating a need for sleep as well.

Non-REM sleep is predominated by low-frequency activity and is often considered "deep and restorative," allowing neurons to rest and recover from their daily activity. In humans, non-REM sleep has traditionally been divided into stages I, II, III and IV. Stage I is relaxed wakefulness, a period which shows large reductions in *alpha, beta* and *gamma* EEG activity but in which people will report they were awake if aroused. Stage II is characterized by sleep spindles and K-complexes, short bursts of *beta* or very low *delta* activity, respectively. Recently, stages III and IV have been collapsed and are collectively referred to as slow-wave sleep, a period in which *delta* wave activity dominates.

In non-human animals, non-REM sleep is not always divided into separate stages and is used interchangeably with slow-wave sleep. Some researchers will divide non-REM into stage I and II, with Stage I being more similar to Stage II in humans, and Stage II equating to the slow-wave sleep of human Stage III. In this paper, rat non-REM sleep will be referred to as slow-wave sleep (SWS),

while human non-REM sleep will be termed 'non-REM' if not being identified by its individual stages.

REM Sleep

As previously mentioned, REM sleep involves rapid movements of the eyes and cortical activity very similar to that of wakefulness. In actuality, the speed of eye movements during wakefulness is much faster than that of REM sleep, but during REM sleep the movements are more dramatic and exhibit repetitive loops.

Other than cortical activity and eye movements, REM sleep and wakefulness are very different. One hallmark of REM sleep is complete dysregulation of autonomic functions, including heart rate, breathing rate, cardiac and arterial pressure, and temperature regulation. Additionally, the hippocampal EEG signal is almost entirely made up of *theta* waves during REM sleep, and high-frequency spikes called ponto-geniculo-occipital (PGO) waves travel from the brainstem to the forebrain [14, 15]. Perhaps the most distinguishing sign of REM sleep is the complete loss of muscle tone, resulting in paralysis. The neurobiology of REM sleep and its sleep signs will be reviewed in Chapter Two.

REM sleep is also the period in which our most vivid and emotional dreams occur, leading some to call it "dreaming sleep," although dreams also occur during non-REM sleep. The high emotional tone of REM sleep dreams has led to the proposition that REM sleep is important for emotional memory processing, an idea which is supported by the fact that many affective disorders are accompanied by dysregulations or disturbances of REM sleep.

The Transition from Non-REM to REM Sleep

Although the mechanisms controlling the onset of non-REM sleep have been well investigated, relatively little is known about how the brain controls the onset of REM sleep. For instance, unlike non-REM sleep, there has been little description of the transition into REM sleep. Also, there have been no studies investigating sex differences in this transitional period. The present study aims to develop a description of the transition from non-REM to REM sleep, and to investigate whether males and females have any differences in this stage, which here will be termed *transition sleep*.

CHAPTER TWO

LITERATURE REVIEW

There are three important concepts that require extensive review in order to fully understand the purpose and consequences of this study: 1) why studying sleep is important, 2) the implications of sex differences in sleep, and 3) what is already known about the transition from non-REM to REM sleep. Here, the literature on those topics will be reviewed and synthesized.

The Function of Sleep

As previously mentioned, the importance of sleep is indicated be its prevalence and uniformity. However, the question of why it is important still remains. Logically, sleep is important because of the benefits it provides for activity during wakefulness. If sleep was not essential for waking activity, animals would not be so affected by sleep loss. So, when the functions of sleep are discussed, it is the functions of sleep as they relate to waking activity that.

Many such functions of sleep have been proposed, but to date there has been no consensus [16]. However, the suspected functions of sleep can be broadly grouped into three categories: hormones & metabolism, immune function, and cognition [17-20]. The focus of the review will be on the cognitive function of sleep, because the cognitive function of sleep is most relevant to behavioral neuroscience and neuropsychiatric disorders.

Just as the phases of sleep have been divided into REM and non-REM, the functions of sleep are often separated by these phases as well; it is generally accepted that non-REM and REM sleep have distinct functions [18, 21, 22]. Support for this idea comes from several observations. For one, the ratio of REM and non-REM within total sleep time changes throughout development and aging, indicating a changing need for the functions of both types of sleep [23-26]. Additionally, non-REM and REM sleep show different homeostatic mechanisms [15, 21, 27] . A homeostatic need for non-REM sleep does not translate to a homeostatic need for REM sleep, and vice-versa. Related to this fact is the observation that different activities during wakefulness produce distinct variations in the different stages of sleep, indicating that each stage serves a function related to distinct activities [18]. On a similar note, total deprivation of sleep produces different effects than selective deprivation of REM sleep, again illustrating that REM sleep serves a distinct function[1] [18, 28-30].

Sleep as a Diagnostic

One important aspect of sleep that has not yet been mentioned is that sleep is a whole-brain process, meaning the entire brain is involved. Although sleep researchers have identified specific areas that are critical for sleep production, maintenance, and homeostasis, that does not change the fact that every part of the brain changes its activity during sleep [31]. This means that anything that

[1] It is impossible for non-REM sleep to be selectively deprived, because non-REM sleep always precedes REM sleep, so any deprivations of non-REM sleep necessarily produce REM sleep deprivations as well.

changes the brain will almost certainly affect sleep as well. Researchers use specific parameters, outlined in Table 2.1, to identify changes in sleep.

It is now widely accepted that sleep and neuropsychiatric disorders share common mechanisms and pathways [17, 32-34]. The links between neuropsychiatric disorders and sleep disturbances allow sleep to serve as a diagnostic for these disorders. Additionally, the distinct sleep changes that are present in different neuropsychiatric disorder can provide insights into the function of sleep. The function of sleep will thus be discussed along these lines: first, what are the sleep disturbances that appear in neuropsychiatric disorders, and second, how do those links relate to the functions of sleep.

Table 2.1. Parameters to Measure Sleep Quality

Measurement	Definition
Sleep Latency	The time it takes for a person to fall asleep after they get in bed.
Sleep Efficiency	The ratio of the amount of time spent asleep compared to the amount of time spent in bed.
Sleep Continuity	Measured by average sleep episode duration and number of awakenings after the first episode of non-REM sleep.
Total Sleep Time	The total amount of time spent asleep in a single night.
Slow-Wave Activity	The power of *delta* frequencies during slow-wave sleep. Higher power is considered better quality.
REM Sleep Latency	The time in between the start of the first non-REM sleep episode and the start of the first REM sleep episode.

Neuropsychiatric Disorders and Sleep

Insomnia is the most commonly diagnosed sleep disorder and is defined as a reduced total sleep time resulting in feelings of daytime sleepiness and fatigue [34]. Approximately 40% of patients diagnosed with insomnia are also diagnosed with a neuropsychiatric disorder, and insomnia or other sleep and circadian disorders are present in nearly 80% of severe mental illness [17, 34]. While a causal link between sleep disruption and mental illness has not been proven, animal models with genetic alterations of circadian genes have altered sleep and some of the behavioral symptoms of neuropsychiatric disorders [33]. Additionally, sleep and circadian changes often precede symptoms of other neuropsychiatric conditions [17, 32, 33]. Here, the sleep changes present in mood disorders, anxiety disorders, neurodegenerative disorders, and psychosis will be reviewed.

Mood disorders are disorders in which a person's mood is overreactive, becoming elevated or lowered to a debilitating extent. The mood disorders are generally comprised of depression, mania, and bipolar disorder, with subclasses that specify the timing of each. Insomnia is present in ≥50% of patients with mood disorders and is usually characterized by the inability to maintain sleep; patients have poor sleep continuity and have long periods of wake during the night [17, 32]. Classically, mania and manic episodes are associated with a decreased need for sleep and short

sleep periods [32, 33]. However, recent findings suggest that mania is not characterized by a reduced need for sleep, but rather insufficient opportunities to sleep because of changes in activity patterns and circadian phases [35].

Depression exhibits what is widely considered the strongest link between sleep disturbances and mental illness, with some reports of >90% comorbidity [17, 32, 33]. In many cases of major depression, there is a marked decrease in Stage III non-REM sleep. However, the changes in REM sleep are considered the most characteristic of depression, because they are not found in other neuropsychiatric disorders [22, 36-39]. There are numerous reports of decreased REM sleep latency [36, 37, 39]. Additionally, many patients with depression experience more REM sleep during the first part of the night – a reversal from the normal pattern, which includes more non-REM sleep in the first half of the night and more REM sleep in the latter half of the night [37-39]. Patients with depression show an increase in the percentage of time spent in REM sleep, although this does not always indicate higher total time spent in REM sleep. Rather, depressive patients tend to have a shorter total sleep duration with an unchanged amount of REM sleep, which increases the ratio of time spent in REM sleep. The strong links between depression and REM sleep suggest a key role of REM sleep in emotional regulation [22, 40].

Anxiety-related disorders do not always involve mood changes and instead are specifically marked by feelings of fear, anxiety or panic at inappropriate times, with disruptions in daily functioning, personal relations, and general well-being. These disorders include generalized anxiety disorder, obsessive-compulsive disorder, panic disorder, phobias, and post-traumatic stress disorder (PTSD). Like mood disorders, insomnia is present in ≥50% of patients diagnosed with an anxiety or related disorder [17]. However, the comorbidity of insomnia and anxiety disorders is often marked by difficulty falling asleep rather than problems staying asleep [17, 41].

Studies have found many different, and sometimes contradictory, changes in sleep quality and architecture in anxiety and related disorders [41]. The most consistent findings are alterations in non-REM sleep architecture, most commonly with reductions in Stage III (slow-wave) sleep [17, 41]. Increases in the percentage of time spent in Stage II is also common [42-46]. No conclusive changes in REM sleep have been identified, although changes in REM sleep are often observed in PTSD [22, 41]. However, there is no consensus on the nature of these changes; many studies find a reduced percentage of time spent in REM sleep due to a longer REM sleep latency and shorter episode durations, while other studies find an increase in the percentage of time spent in REM sleep [47-49]. Of note, PTSD involves more severe emotional distress than other anxiety disorders, so these REM sleep alterations could be more indications of the relationship between REM sleep in emotional regulation [22]. Altogether, slow-wave sleep appears to have the most conclusive role in managing anxiety, fear, and panic.

The neuronal mechanisms of mood and anxiety disorders are not precisely understood, unlike the neurodegenerative disorders, which by definition involve a progressive loss of neurons, though there is a wide variety of causes for this degeneration. This category of disorders includes Alzheimer's disease, Parkinson's disease, Huntington's disease, dementia with Lewy bodies, and multiple system atrophy. The sleep changes present in these disorders somewhat resemble the changes that occur with normal aging [25, 50]. Particularly, neurodegenerative disorders often produce fragmented sleep, reductions in the percentage of time spent in Stage III sleep, and increases in time spent in Stage I sleep [50]. Additionally, the EEG features of Stage II sleep are shorter in duration, occur at a lower frequency and are less well-formed.

In normal aging, REM sleep remains relatively stable until very old age. However, in neurodegenerative disorders, several changes in REM sleep are observed. Most commonly, a reduction in REM sleep occurs due to shorter REM sleep episode durations [51-56]. Additionally, there may be a slowing of cortical EEG wave frequencies during REM sleep, meaning that cortical EEG signals contain a higher proportion of slower frequencies such as delta and theta, with reductions in alpha and beta frequencies [56-63]. The severity of Alzheimer's disease is particularly well correlated to the slowing of cortical EEG in the temporal regions [64-66].

Some neurodegenerative disorders are often preceded by REM sleep behavior disorder (RBD), which is characterized by the absence of normal paralysis during REM sleep and involves complex motor activity relating to dreaming during REM sleep [50, 67]. In particular, the diseases that involve abnormal aggregation of alpha-synuclein protein, which include Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy, are linked to RBD [50]. The symptoms of RBD appear to be indicative of disinhibition of spinal motor neurons and various behavioral systems, due to neuronal cell loss [68].

Altogether, the characteristics of sleep in the neurodegenerative disorders point to the aspects of sleep that seem to most relate to cognition: the sleep spindles and K-complexes of Stage II sleep, the slow-waves of Stage III sleep, cortical activation during REM sleep, and adequate amounts of sleep in general.

Psychosis is not a distinct category of disorders, but instead is a feature present in some neuropsychiatric disorders, such as depression with psychotic features, hallucinations in Parkinson's disease, or schizophrenia. The word psychosis is used to describe thoughts and emotions that are so impaired they cause some loss of contact with external reality. Symptoms of psychosis include delusions (false beliefs), hallucinations (seeing or hearing things that others do not see or hear), incoherent or nonsense speech, and behavior that is inappropriate for the situation.

Psychosis has been found to have strong links to REM sleep; REM sleep without loss of muscle tone is linked to psychosis [17, 69-71]. Generally, this muscle tone does not result in full body movements, as is seen in RBD, but is related to more periodic limb movements and sudden muscle contractions. Although this does not have to reach the levels of RBD, the presence of RBD is significantly correlated with visual hallucinations in Parkinson's disease [70, 71]. One interesting possibility is that psychotic episodes are actually REM sleep "dreaming" activity that occurs during or interferes with wakefulness [72, 73].

Additionally, psychosis is found to correlate with "disassociated" stages of sleep, in which the components of certain sleep stages appear in other stages of sleep, or there are rapid oscillations between sleep stages [17, 69, 72, 73]. This rapid oscillation often results in very fragmented sleep as well. Overall, the sleep patterns that appear linked to psychosis are referred to as "sleep instability," and illustrate a brain system that struggles to fully differentiate stages of sleep and wakefulness [69, 74]. Sleep in psychosis offers a dramatic view of the ways in which sleep changes can offer insights into the neural dynamics of psychiatric disorders. For example, schizophrenia is known to be linked to altered brain circuitry, which could explain alterations in the organization of sleep stages [75-77].

Although the disruptions in sleep found in each of these neuropsychiatric disorders may appear distinct, important similarities exist. The ratio of non-REM to REM sleep across the night is varied in each disorder, either by reductions in some of the characteristics of non-REM sleep stages or by changes in the timing or characteristics of REM sleep. In light of this, it may not be ideal to focus on the distinct functions of non-REM and REM sleep, but rather to focus on how they interact to optimize the brain's waking functionality.

The Cognitive Function of Sleep

Sleep appears to be critical for multiple aspects of cognition, including attention, , judgement, evaluation, decision making, emotional regulation, learning and memory [2, 18, 22, 78-81]. While deprivation studies and observations in neuropsychiatric disorders have consistently shown this to be true, causal mechanisms are still out of reach [16, 17, 22]. However, several features of sleep have been selected as particularly important for cognition. In non-REM sleep, the spindles that appear in the cortical EEG during Stage II have been shown to have a strong correlation with cognitive function [18, 30, 69, 82-84]. Additionally, the slow-waves that appear in the cortical EEG during Stage III are also considered important [83, 85, 86]. In REM sleep, the theta activity in the hippocampus as well as the activated cortical EEG have been identified as crucial for cognition and emotional memory [8, 18, 87].

These findings support two separate possibilities for the mechanisms by which sleep promotes memory consolidation. One hypothesis is that global synaptic downscaling during sleep optimizes the brain for functioning the next day, and memory consolidation is a byproduct of that process. Another hypothesis is that selective reactivation of memories during sleep promotes the redistribution of connections within the neocortex, incorporating new information into preexisting "knowledge networks." Of course, these two hypotheses are not mutually exclusive and both processes likely contribute to memory consolidation [18].

Non-REM sleep is the most likely candidate for processes involved in the synaptic downscaling hypothesis. Slow-waves in particular seem fit for long-term depression, or depotentiation of synapses, which facilitate the encoding of new information after sleep [88, 89]. In fact, the slow oscillations of depolarization and hyperpolarization that produce slow-waves are associated with activation of T-type Ca^{2+} channels, which seem to favor long-term depression [90]. This is congruent with the reductions of slow-wave sleep found in aging, dementia, and neurodegenerative disorders. Additionally, the reductions of slow-wave sleep seen in anxiety-related disorders is interesting and could provide insight into the neurobiology of anxiety.

As for the reactivation hypothesis, this involves more harmonious roles of both non-REM and REM sleep. The previously mentioned spindles that appear in Stage II may act to prime specific neuronal networks for transfer from the hippocampus to the cortex [18, 91, 92]. These spindles involve continuous, synchronized communication between the cortex and the thalamus and occur in parallel to reactivation of memory traces in hippocampal circuits, which produce their own ripples of high-frequency activity in the cortex during slow-wave sleep [93-95]. The redistribution of memories from the hippocampus to the cortex is a necessary step in the formation of long-term memories, and human imaging studies have provided evidence for a role of sleep in this process [96-99].

However, redistributing memory traces is not enough to strengthen and stabilize the synaptic connections that underlie long-term memory. REM sleep, on the other hand, has several mechanisms to accomplish this task. For one, the PGO waves that occur prior to and during REM sleep project to the hippocampus, cortex, and amygdala, and have a frequency high enough to induce long-term potentiation [14, 100]. Additionally, REM sleep is associated with an upregulation of plasticity-related immediate early genes [101, 102]. Ultimately, cortical activity during REM sleep is high but not coherent between different regions, creating an environment in which local synaptic plasticity is amplified and almost entirely unbiased by external inputs [103, 104]. Coupled with the transfer of information between brain regions that occurs during slow-wave sleep, this is the perfect environment to create stable long-term memories and knowledge networks [18].

REM sleep is also postulated to play a strong role in emotional regulation [22, 38, 97]. As previously stated, this is most commonly driven by the association between mood disorders and REM sleep [39]. The relationship between emotional regulation and REM sleep has traditionally been attributed to the neurochemical profile of REM sleep: low aminergic tone and high cholinergic tone [105]. Since the monoamines are more associated with emotional activity, this profile allows for information processing with little emotional activity and allows the brain to "reset" its levels of monoamines for optimal emotional processing [22, 40]. However, there are several problems with this theory. One, it does not explain why drugs that increase monoamines are used to treat depression, or why those drugs take weeks to have a therapeutic effect [22, 39]. Additionally, it does not account for the high emotional tone of REM-sleep dreams.

The newer concept of depression as a disorder of neuronal plasticity points to the involvement of REM sleep because of its plasticity-related functions. One potential hypothesis that has not been well investigated is that a dysregulation of the cycling between non-REM and REM sleep may be an underlying factor in depression and other neuropsychiatric disorders. With the cooperative natures of non-REM and REM sleep, optimal network formation would occur when the role of non-REM sleep has been adequately fulfilled before the first REM sleep episode. This may be reflected in the normal human sleep cycle, which involves more non-REM sleep, particularly slow-wave sleep, in the first half of the night while REM sleep predominates in the second half. Disruptions or alterations to this cycle could thus result in REM sleep occurring before the brain has been adequately prepared, resulting in suboptimal network formation.

Sex Differences in Sleep

There is emerging research and clinical evidence that there are sex differences in sleep and sleep regulation [106, 107]. Research into sex differences in sleep is in its infancy, and there are mixed findings [108]. Compared to men, women and girls worldwide are twice as likely to experience sleep disruptions and have a 40% greater risk for developing insomnia at some point in their lifetime [109, 110]. However, one consistent finding is that healthy women have better objectively-defined sleep quality than men [111-116]. On average, women have significantly longer total sleep time, spend less time awake while in bed, and fall asleep faster after getting in bed [112, 113, 115]. Additionally, women have more Stage 3 sleep, have a higher amount of slow-wave activity within Stage 3 sleep, and have slow-wave activity that is less affected by aging than men [115, 117-120].

However, women consistently report more disrupted and insufficient sleep than men in self-assessments, suggesting there is a disconnection between objective sleep quality and subjective sleep quality in women [121-124]. It is unclear why this discordance occurs; one possibility is that traditional measures of sleep quality have been based on male physiology and may not accurately measure sleep quality in women [108, 125]. Alternatively, self-report biases may be responsible for these differences.

In men, testosterone secretion is tightly linked to REM sleep, with peak levels occurring just before or after REM sleep [126]. However, there are several lines of evidence that indicate women are more sensitive to the effects of sex steroids on sleep. Sleep disruptions in women begin to occur during puberty and are linked to fluctuations in ovarian steroids [127-129]. Sleep complaints also accompany other periods of sex steroid fluctuations, such as the menstrual cycle, pregnancy, and menopause [128-133]. Importantly, some of these changes may be due to physical changes and not to direct effects of sex steroids. More research on the influence of ovarian steroids on sleep is needed [108].

Animal research has produced some evidence supporting the trends found in humans. For example, in mice, females are found to have less total sleep and non-REM sleep than males but have longer and more consolidated sleep episodes with fewer transitions between states and more slow-wave activity [134-136]. These differences appear to agree with the differences observed in humans, and disappear when circulating sex steroids are removed, suggesting they are dependent on sex steroids.

Research in rats also supports the claim that sleep in females is more sensitive to fluctuations of sex steroids than in men. Gonadectomized male and female rats exhibit no significant differences in sleep-wake activity, but exogenous replacement of sex steroids results in a significant reduction of all sleep stages during the dark phase in females but not males [134, 137, 138]. Importantly, rats are nocturnal, so these results indicate that estradiol may act to consolidate sleep in the appropriate circadian phase. Sleep in female rats is tightly linked to fluctuations of ovarian steroids over the estrous cycle; during pro-estrous, when estradiol and progesterone are both elevated, nocturnal non-REM and REM sleep are suppressed [139-141]. Exogenous estradiol and progesterone are sufficient to reproduce these changes in ovariectomized female rats [139, 142, 143].

In male rodents, sleep seems insensitive to changes in levels of sex steroids. Castration does not significantly change sleep-wake activity, and administration of estradiol has no effect on sleep in male rats [108, 137]. This raises the possibility that sex differences in sleep could in part be due to the organizational effects of sex steroids [137, 144, 145]. Female rats exposed to a masculinizing dose of testosterone during the sensitive window for brain sexual differentiation exhibit male-like insensitivity to sex steroids [137]. Additionally, the sleep-promoting neurons in the ventrolateral preoptic area exhibit male-like patterns of activity in masculinized females, illustrating that a component of sleep circuitry is sensitive to sex steroids [137, 140]. The lateral hypothalamus, another area involved in sleep regulation, seems sensitive to fluctuations of ovarian, but not gonadal steroids [143, 146, 147]. Altogether, evidence suggests that females may possess sleep-regulating brain circuitry that is less rigid than that of males, and these differences could be regulated by sex hormones.

Importantly, the differences in sleep between the sexes do appear to have mental and physical health consequences. Women appear to experience the effects of sleep loss more quickly than men do, and the consequences of sleep loss seem to be more debilitating [106]. In fact, studies have shown that restricting sleep puts women at a higher risk for developing hypertension, cardiovascular problems, and metabolic disorders [106]. The occurrence of insomnia in women is associated with a twofold greater risk for depression than that of men with insomnia [106, 108, 125]. In patients with major depression, females show greater changes in sleep quality than men [148]. Women tend to have more disrupted sleep after exposure to a trauma and are at greater risk for developing PTSD. Additionally, women who develop PTSD after a traumatic event have more disrupted sleep than women who don't [149, 150]. It is obviously imperative that there are more investigations into the causes, effects and treatments of sleep disorders in women.

Transition Sleep: What We Know

The neural generation and homeostatic regulation of REM sleep has been well-studied. It is widely accepted that neurons in the dorsolateral pons are necessary and sufficient for producing REM sleep [12, 15, 105]. These neurons are cholinergic, and REM sleep is neurochemically characterized by high levels of acetylcholine throughout the brain, particularly in the cortex, with low levels of monoamines and GABA [12, 105, 151]. Retro- and anterograde tracing has shown that neurons in the dorsolateral pons have connections with the brain regions that collectively produce the signs that are associated with REM sleep: paralysis, autonomic dysregulation, rapid eye movements, theta waves in the hippocampus, and an activated cortex [15].
It has long been hypothesized that there are reciprocal interactions between aminergic REM-off cells and cholinergic REM-on cell groups which regulate the non-REM/REM sleep cycle. More recent versions of this hypothesis recognize that there are GABAergic and glutamatergic cell populations that also promote or inhibit REM sleep [152]. Our basic understanding of REM sleep control hinges upon brain regions with reciprocal inhibitory projections that produce a "flip-flop switch" [153]. This means that the control of REM sleep is entirely dependent on whether or not the REM-promoting region overcomes the inhibition of the REM-inhibiting region. Ultimately, however, exactly how this occurs is not well understood [154, 155].

Although the functions of non-REM and REM sleep are often segregated, it has also been proposed than REM sleep serves a function that is reactionary to non-REM sleep [21]. The crux of this argument is that REM sleep occurs not for the benefit of waking activity, but for the benefit of non-REM sleep. Proponents of this claim point to the fact that REM sleep does not occur until an adequate amount of non-REM sleep is achieved, and non-REM sleep recovery is always prioritized over REM sleep recovery if both types of sleep are deprived [21]. While the field seems to have come to a consensus that REM sleep does serve its own separate function for wakefulness, the argument points out something which models of non-REM/REM sleep cycling seem to ignore: the timing of REM sleep seems to be a function of non-REM sleep and not of activity during wakefulness [14, 154]. How and why, exactly, does activity during non-REM sleep relate to the REM sleep "switch"? We do not know.

The idea that a period between non-REM and REM sleep is a separate sleep state was explored by the French researcher Claude Gottesmann in the 1990's [156]. Gottesmann described this state in rodents as characterized by slow-wave, high amplitude spindles in the cortical EEG accompanied by

theta activity in the hippocampus. In humans, there is usually a short period of what is categorized as Stage II sleep immediately before REM sleep, which Gottesmann claims is in fact transition sleep. Interestingly, a few studies found this sleep stage to be absent in dementia but extended in patients experiencing psychosis [156].

Gottesmann conducted a number of studies investigating the neurobiology of transition sleep [156]. He found that transition sleep was unaffected by ritanserin, a serotonin antagonist that inhibits REM sleep. Additionally, barbiturates seemed to extend transition sleep while suppressing REM sleep. These data suggest transition sleep has a mechanism independent of REM sleep. However, atropine, an anticholinergic drug, decreased both transition sleep and REM sleep, suggesting they share a cholinergic mechanism. Ultimately, Gottesmann abandoned the investigation of transition sleep after numerous attempts to uncover the neuroanatomical and/or neurochemical substrates of transition sleep failed to generate a cohesive understanding.

Some recent studies have begun to investigate transition sleep, but do not qualify a distinct sleep stage *per se* and instead investigate how the number of transitions relates to other qualities of the stages of sleep [157]. New information on transition sleep comes from investigations of the spindles that occur during slow-wave sleep, which Gottesmann believed were a sign of transition sleep. There are a few studies which link these spindles to cognitive function, and the expression of immediate early genes induced by REM sleep has been linked to the amount of spindle activity in the immediately preceding slow-wave sleep [18, 102]. One study showed that LTP at the SWS/REM transition critically influences the effect of sleep on synapse structure: “Its lack determines synaptic homeostasis; its presence causes synaptic restructuring.” [155]. Those authors concluded that slow-wave sleep and REM sleep have synergistic roles in cognition and point to the stage between non-REM and REM sleep as serving its own distinct role.

Although these studies provide evidence for the existence of a functionally distinct sleep stage between non-REM and REM sleep, investigations into this sleep stage still remain limited. With the modern understanding of non-REM and REM sleep serving cooperative roles, it is more important than ever to understand the relationship between the two sleep stages, including why, how, and when the brain transitions from one to the other. Understanding the mechanisms of transition sleep is crucial for understanding this relationship. However, before neurobiological mechanisms can be elucidated, a more complete behavioral description of transition sleep is needed in both males and females. There is ample evidence that there are sex differences in sleep structure and quality, including the timing and perturbation of REM sleep. Because transition sleep is thought to regulate the relationship between non-REM and REM sleep and sex differences exist in sleep patterns, we expect male and female rats to differ in the expression of transition sleep. Overall, the aim of this study is to identify sex differences in transition sleep and provide a novel metric for sleep quality. We expect this descriptive study to lay the foundation for future studies focused on the neurobiology of transition sleep and the mechanisms by which psychiatric disorders alter sleep.

CHAPTER THREE

MATERIALS AND METHODS

Subjects and Housing

Subjects

The subjects were 8 adult male and 8 adult female Sprague-Dawley rats weighing between 250 – 300 g. All experimental procedures were performed in accordance with IACUC regulations (IACUC protocol #2311, #2349, & #2565) and NIH guidelines. To minimize the stress of experimenter handling, animals were habituated to human contact with 5 – 10 minutes of daily handling for one week and every weekday for the duration of the study.

Four animals had to be removed from the study early due to loss of implants that were necessary for data collection. The final number of subjects included in data analysis was 6 male and 6 female rats.

Housing

Subjects were individually housed in a sound-attenuated room with a 12-hour light cycle (lights off at 7 PM, lights on at 7 AM), in 12 in. x 12 in. clear acrylic cylindrical cages (Pinnacle T) filled with cobb bedding to a depth of 1 – 1 ½ in. Subjects were housed individually to prevent grooming and accidental removal of the implants. Food and water were provided ad libitum. The food was kept in stainless steel bowls within the cage, which were refilled every 2 – 3 days. Water was kept in glass water bottles with drip-proof stainless steel spouts and was also changed every 2 – 3 days.

Surgical Procedures

Preparation for Surgery

One week after arrival in the housing facility, animals were subjected to an intracranial surgical procedure. The scalp of each animal was shaved, and animals were then placed in a stereotaxic apparatus, and secured the head with blunt rodent ear bars. Anesthetic depth was maintained by continuous delivery of isoflurane at concentrations between 0.5-2%. The appropriate depth of anesthesia was determined by the absence of response to a foot pad pinch. Body temperature was maintained using a far-infrared heating pad (Kent Scientific, DCT-20).

The scalp was cleaned with alternating scrubs of an iodine solution and 90% ethanol. Once clean, subcutaneous injections of lidocaine (5 mg/kg) and atropine (0.04 mg/kg) were administered under the scalp, to minimize pain. Then, a portion of the scalp was removed to reveal the frontal and parietal skull bone plates. The first layer of the skull bones was gently scratch off, to help create a dry field for dental acrylic to adhere to. Then, the skull was cleaned with hydrogen peroxide and 90% ethanol and allowed to dry. Potential postoperative pain was preemptively controlled with buprenorphine (s.c., 0.05 mg/kg).

Intracranial Implantation of Electrodes

To identify sleep-wake states, it is necessary to have electrophysiological signals from the cortex, hippocampus, and neck muscles. To enable this, electroencephalogram (EEG) and electromyogram (EMG) electrodes were implanted. To record cortical EEG, small holes were drilled into the skull 2.0 mm anterior and 3.0 mm lateral to bregma. Stainless steel screw electrodes connected to Teflon-coated stainless steel wires (Plastics1) were screwed into these drill holes. An additional electrode was screwed into the skull (3 mm posterior and 3 mm lateral to the bregma in the midline) to act as a ground electrode.

To record hippocampal EEG, a small hole was drilled into the skull 3.8 mm posterior and 2.5 mm lateral to bregma. After gently tearing the dura under the drilled hole, a stainless steel electrode coated in Formvar (California Fine Wires) was stereotaxically implanted at a depth of 4.0 mm. A pair of Teflon-coated stainless steel wire electrodes were implanted bilaterally for recording neck muscle EEG. All electrodes were secured to the skull with dental acrylic and crimped to pins which were then placed in a plastic connector (Plastics 1). The plastic connector was then also secured with dental acrylic, forming the head cap.

Surgical Recovery

Immediately after surgery, animals were removed from the isoflurane and were given carprofen (s.c., 5 mg/kg) to minimize postoperative pain and reduce swelling and inflammation. Animals were kept on the heating pads until they regained consciousness. Then, animals were returned to their cages and recovery was monitored for 2-4 hours. Successful recovery was gauged by the return of normal postures, voluntary movement, and grooming. At this point animals were transferred to their normal housing facility, and their condition was checked daily. Any pain was treated with buprenorphine and swelling/inflammation was treated with bacitracin and carprofen. After the surgical site was relatively healed, with no visible bleeding, excess inflammation, or infection, rats were habituated to the sleep-wake recording conditions for 3-5 days.

Sleep-Wake Recordings

Recording Conditions

A three-channel biopotential recording system (Pinnacle Technology) was used to record animals' sleep-wake activity. During habituation to the setup and during the sleep-wake recordings, animals were allowed free movement in their cages while connected to a low-torque swivel by a counterbalanced cable that ensures minimal strain on the animals' heads. At one end of the cable, there are pins that connect the cable to the rats' head caps. Above these pins there is a preamplifier that amplifies the signals by 10X. At the other end of the cable, there are pins that connect to the swivel, which is connected to a commutator. The signals are sent to the commutator for conditioning and final-stage amplification and filtering. The cortical and hippocampal EEG signals were sampled at 1kHz, and band-pass filtered between 0.5 Hz and 100 Hz. The EMG signals were sampled at 2kHz, and band-pass filtered between 10 Hz and 200 Hz.

From the commutator, the signals were passed to a computer located in the next room. From this computer, it was possible to monitor the rats without disturbing them. This provided access to the rats during their 24-hour recordings without disturbing their sleep-wake cycles.

Recording Procedure

Recordings were taken every 4 days for 2 weeks (Days 1, 5, 9 and 13). On recording days, animals were connected to the recording system at 8 AM. Recordings began at 10 AM, allowing enough time for the animals to readjust to the connecting cable. During this time, animals' food and water was changed as necessary. No recordings were taken within 24 hours of a cage change, since new cages are known to disrupt rodent sleep-wake cycles [158].

Animals remained connected to the recording system for 24 hours (until 10 AM the following day). After recordings were finished, animals were disconnected from the recording system to prevent any unnecessary stress or strain on the animals' head caps.

Data Analysis

Scoring Sleep-Wake States

After the 24-hour recordings were collected, the sleep-wake data were analyzed and scored for four states: Wakefulness, Slow-Wave Sleep (SWS), REM sleep (REMS), and Transition Sleep (tRS). These four states are described in Table 3.1. The 24-hour recordings were scored in 6 second time increments (bins), to ensure high resolution of tRS episodes, which are relatively short. Simultaneous recordings of cortical EEG, hippocampal EEG, and neck muscle EMG ensured accurate identification of behavioral states in this study.

Table 3.1. Criteria for Scoring Each of the Four States of Sleep and Wakefulness

Stage	Cortical EEG signal	Hippocampal EEG signal	Neck muscle EMG signal
Wake	Low-amplitude (50-80 μV) and fast (30-100 Hz)	Mixed, tends to be higher frequency than SWS	High-amplitude phasic and tonic bursts
Slow-Wave Sleep	High-amplitude (200-400 μV) and slow (0.5 – 15 Hz)	Mixed, tends to be lower frequency than wake	Low tone with few bursts of activity
Transition Sleep	A mixture of low-amplitude, fast waves and high-amplitude, slow waves. Some spindle-like activity.	Low theta frequency (8 – 10 Hz) appears.	Absent or progressively diminishing
REM Sleep	Similar to Wake	Very saturated, continuous theta frequency (8 – 14 Hz)	Completely absent (atonia)

Analyzing Sleep-Wake Data

The scores of the 24-hour recordings allowed for the quantification of the following variables for each recording session: (1) the percentage of time spent in W, SWS, tRS, and REMS, (2) total number of SWS, tRS and REMS episodes, and (3) average duration of SWS, tRS and REMS episodes. The quantification of these variables was divided between the rats' light (inactive) period (7 AM – 7PM) and dark (active) period (7 PM – 7 AM), to prevent the results of the active period from influencing the results of the inactive period, and vice versa.

Percentages were calculated as total time (in secs) spent in each state in the light or dark period, divided by the total time spent in each period (12 hours each; 43,200 secs). For each animal, an episode was a sequence of bins that were scored as a single state and were uninterrupted by another state. For wakefulness, SWS and REMS, the minimum number of bins necessary to be considered an episode was two (min = 12 secs). This prevented very brief (≤ 6 secs) bouts of these states from skewing their number and average duration of episodes. For tRS, because tRS episodes are considerably briefer than the other states, the minimum number of bins necessary to be considered an episode was one (min = 6 secs). Average durations were calculated as total time spent in a state divided by the number of episodes of that state.

Additionally, an hour-by-hour analysis was used to quantify the percentage of each hour spent in SWS, REMS, and tRS. In this analysis, percentages were calculated as total time (in secs) spent in each state for each hour, divided by one hour (3600 secs). The number of episodes and average duration of episodes for each state in each hour was also calculated.

Statistical Analyses

For statistical analyses of light and dark periods, each variable was analyzed by a two-way (sex × day) ANOVA with repeated measures (RM-ANOVA). In the figures, all four recording days were averaged when no effect of day was found in the two-way RM-ANOVA. In each group, data were missing from one animal on one day (4.17% of total data) due to malfunctions with the recording system or accidentally setting recording durations that were shorter than 24 hours. Thus, the total number of data points for each group was 23, not 24. For two-way RM-ANOVAs, the missing data points were estimated by using the average of the other 5 animals for that day. However, the figures show data without estimating missing values.

For the hour-by-hour analysis of the percentage of time spent in tRS, the data were averaged over the four sampling days, resulting in 23 recording events for both sexes averaged in each hour of the analysis. To investigate the relationships between sleep stages, all hourly variables were used to create linear regression models of the hourly percentage of time spent in SWS, tRS, and REMS.

Mauchly's Test of Sphericity was used to test for homogeneity of variance in all conditions of the RM-ANOVAs. When sphericity was violated, the Greenhouse-Geisser correction was applied. Significance threshold was set at $\alpha = 0.05$, $p < 0.05$. In the figures, error bars represent the standard error of the mean.

CHAPTER FOUR

RESULTS AND DISCUSSION

The presentation of results emphasizes sex differences and collapses across the four sampling days because there were no significant effects of day, except where indicated. For an overview of sleep-wake activity across a 24-hour recording, a representative hypnogram for a male and female rat are presented in Figure A.1.

Wakefulness and Slow-Wave Sleep were Not Moderated by Sex

No significant differences between the sexes were found in the percentage of time spent in wakefulness in either the light or dark period (light: $F_{(1,10)}=1.02$, $p > 0.05$; dark: $F_{(1,10)}=2.35$, $p > 0.05$; Figure 4.1). This finding indicates that sex did not influence the amount of time spent awake. Wakefulness was not further investigated in this study, because it can be indirectly measured by quantifying SWS (wakefulness and SWS episode numbers are always similar because wakefulness almost always precedes SWS but almost never precedes tRS or REMS). Also, wakefulness is unlikely to affect tRS beyond changing the amount of total sleep time.

There were also no significant differences between the sexes in the percentage of time spent in SWS (light: $F_{(1,10)}=2.49$, $p > 0.05$; dark: $F_{(1,10)}=0.01$, $p > 0.05$), the number of SWS episodes (light: $F_{(1,10)}=3.24$, $p > 0.05$; dark: $F_{(1,10)}=0.43$, $p > 0.05$), or the average duration of SWS episodes (light: $F_{(1,10)}=3.9$, $p > 0.05$; dark: $F_{(1,10)}=0.63$, $p > 0.05$). Collectively, the data indicate that sex did not influence the amount or quality of SWS (Figures 4.1, 4.2 and 4.3).

Sex Differences in Transition Sleep

Statistical analyses revealed significant sex differences in the percentage of time spent in tRS ($F_{(1,10)}=6.73$, $p < 0.001$) as well as number of tRS episodes ($F_{(1,10)}=10.252$, $p < 0.01$) during the light, but not the dark phase. Pairwise comparisons showed that females had a higher average percentage of time spent in tRS ($mean_M=0.025$, $mean_F=0.033$) and a higher average number of tRS episodes ($mean_M=6.78$, $mean_F=8.50$; Figures 4.1 and 4.2). The duration of tRS episodes did not vary between sexes (light: $F_{(1,10)}=0.10$; dark: $F_{(1,10)}<0.01$; Figure 4.3). Altogether, females appeared to have a higher number of tRS episodes in the light phase, which resulted in a higher percentage of time spent in tRS compared to males.

Although sex did not have a main effect on the number of tRS episodes in the dark phase, there was a significant interaction between sex and day ($F_{(3,30)}=3.08$, $p < 0.05$). Pairwise comparisons revealed that females had a significantly higher number of tRS episodes than males on Day 1 ($mean_M=3.57$, $mean_F=5.45$; $F_{(1,10)}=5.11$, $p < 0.05$), and a near-significant increase compared to males on Day 5 ($mean_M=2.90$, $mean_F=4.77$; $F_{(1,10)}=4.91$, $p = 0.051$; Figure A.2). There was also a main effect of day ($F_{(3,30)}=4.98$, $p < 0.01$). Pairwise comparisons revealed that the average number of tRS episodes for both sexes was significantly lower on Day 9 (mean=2.49), compared to Day 1 (mean=4.51) and Day 5 (mean=3.83; Figure A.2). However, these differences appear to be due to the variations in the

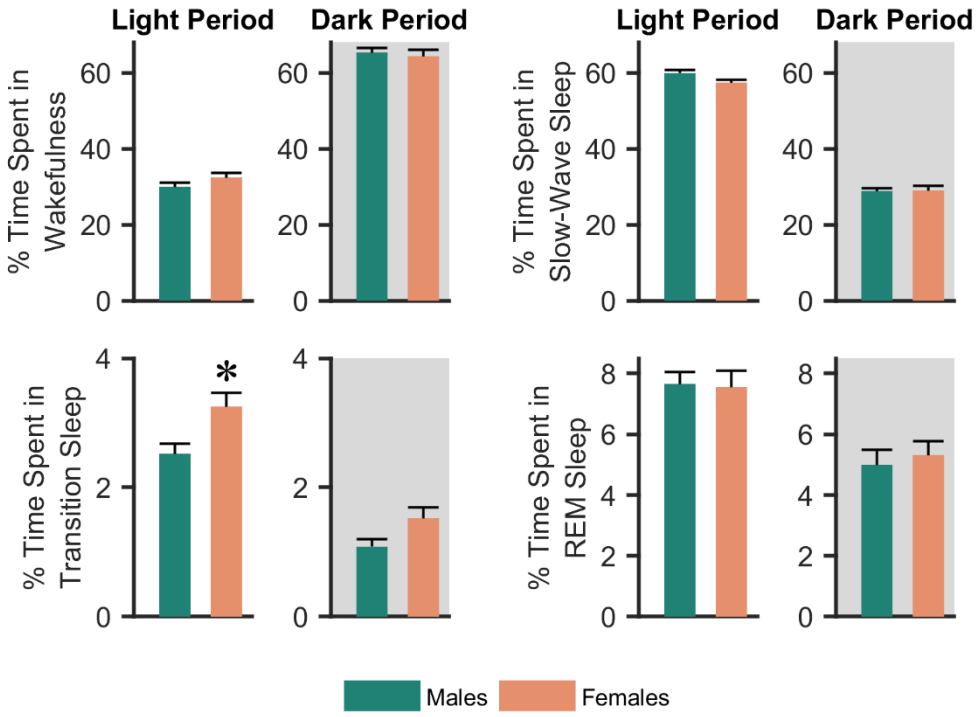

Figure 4.1. Percentage of Total Recording Time Spent in Each State.

The figure shows histograms representing the average percentage of the light and dark periods spent in wakefulness and each stage of sleep for the male and female groups. Note that no significant differences between the males and females were found in any state except transition sleep. The figure indicates that, in the light phase, females had a higher percentage of time spent in transition sleep compared to males. There were no differences between males and females in the dark phase. $* p < 0.05$.

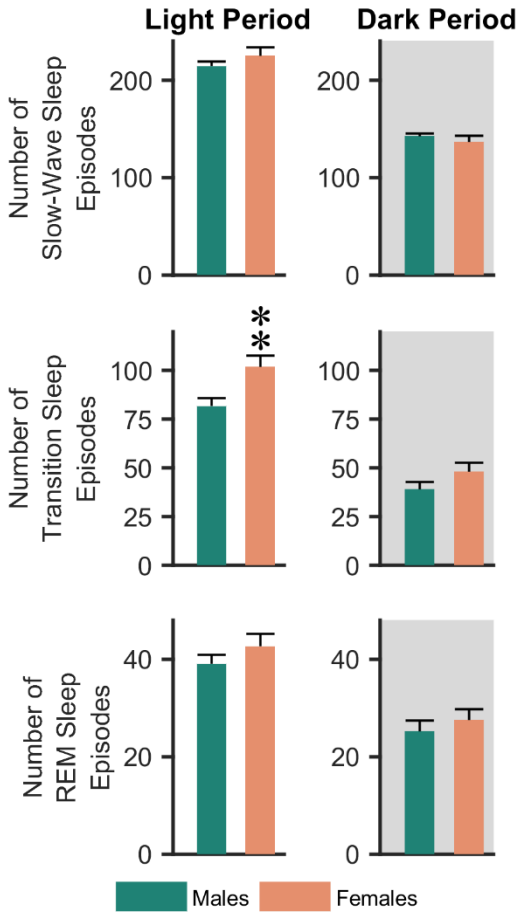

Figure 4.2. Total Number of Episodes of Each Sleep Stage.

The figure shows histograms representing the average total number of episodes of each sleep stage for males and females in the light and dark periods. Note that there were no differences between males and females for any stage of sleep, except transition sleep. The figure shows that, in the light phase, females entered transition sleep at a higher frequency than males. There were no differences between males and females in the dark phase. ** $p < 0.01$.

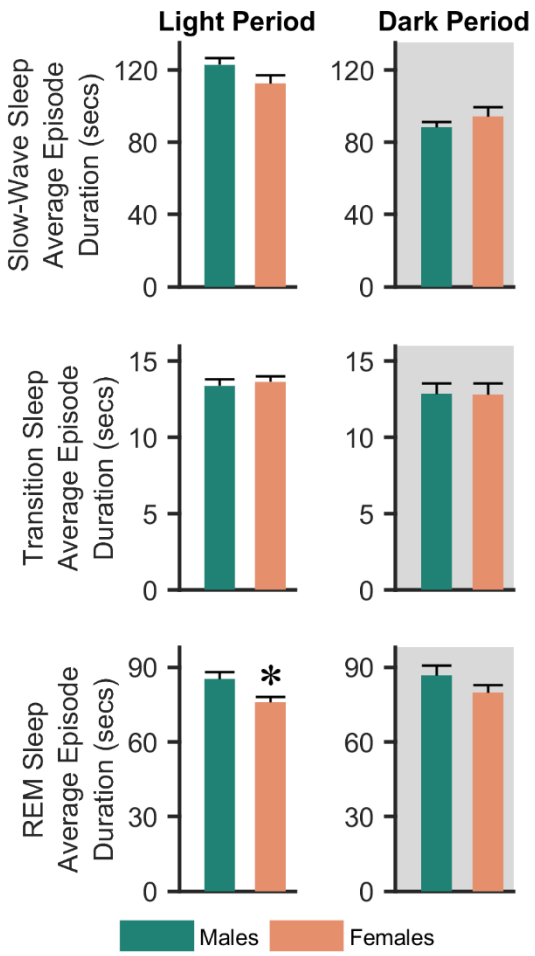

Figure 4.3. Average Duration of Episodes of Each Sleep Stage.

The figure shows histograms representing each group's mean average duration of episodes for each stage of sleep. Note that no significant differences between the males and females were found in any sleep stage, except REM sleep. The figure illustrates that, in the light phase, males exhibited longer average REM sleep episode durations compared to females. There were no differences between males and females in the dark phase. $* p < 0.05$.

females, not the males (Figure A.2). Altogether, the data indicate that females may exhibit high variability in the daily frequency of tRS during the dark phase.

Our RM-ANOVA of the percentage of each hour spent in tRS revealed significant main effects of sex ($F_{(1,44)}$=6.05, $p < 0.05$) and a near-significant sex × hour interaction effect ($F_{(23,1012)}$=17.74, $p = 0.05$). As expected from results of the day-by-day analyses, pairwise comparisons showed that females had a higher average percentage of each hour spent in tRS compared to males ($mean_M$=0.019, $mean_F$=0.024; Figure 4.4). A decomposition of the sex × hour interaction revealed that females spent significantly more time in tRS than males at the hours of 9 am, 12 pm – 1 pm, 4 pm, 12 am, and 4 am (Figure 4). Table A.1 shows the statistics for percentage of time spent in tRS for each hour in males vs. females.

The RM-ANOVA also revealed a significant main effect of hour ($F_{(23,1012)}$=17.74, $p < 0.05$). Noon to four pm seemed to be the peak time for tRS; each hour between noon and four showed elevated transition sleep compared to other times of day. During the dark period, both sexes had a significantly lower percentage of time spent in tRS than during the light period. Table A.2 shows the pairwise comparison statistics for the percentage of time spent in tRS for each hour.

Sex Differences in REM Sleep

There were no statistically significant sex differences in the percentage of time spent in REMS (light: $F_{(1,10)}$=0.01, $p > 0.05$; dark: $F_{(1,10)}$=0.72, $p > 0.05$) or the number of REMS episodes (light: $F_{(1,10)}$=2.06, $p > 0.05$; dark: $F_{(1,10)}$=0.45, $p > 0.05$) in either the light or dark period. However, there was a significant sex difference in average REM sleep episode duration during the light phase ($F_{(1,10)}$=6.03, $p < 0.05$), but not the dark phase ($F_{(1,10)}$=0.01, $p > 0.05$). Pairwise comparisons revealed that females had shorter REM sleep episodes than males ($mean_M$ = 85.25, $mean_F$=76.02; Figure 4.3). This is interesting in light of the fact that females spent more time in tRS sleep and had a higher average number of tRS episodes. It suggests that there is a difference between the sexes in the production and/or regulation of REM sleep. The data support the claim that females enter tRS more readily than males, but this does not translate to an increase in the amount of REMS, and they do not maintain REMS to the same extent that males do.

To further investigate sex differences in the relationship between tRS and REMS, the ratio of tRS episodes that were followed by REMS episodes compared to the total number of tRS episodes was calculated for the total time of each of the four 24-hour recordings. This ratio is an expression of the likelihood that REMS will follow tRS. These data were then analyzed using a RM-ANOVA. There was no effect of day, and no significant sex difference was found, although the ratio was slightly lower for females ($F_{(1,10)}$=0.38, $p > 0.05$; Figure A.3). The ratio for each hour of the four 24-hour recordings was also investigated using a RM-ANOVA. Interestingly, there was a pattern in which males had a higher probability of entering REMS after tRS in the first four hours of the light period, although the RM-ANOVA was not significant ($F_{(1,44)}$=1.06, $p > 0.05$, Figure A.3). Combined, it seems that tRS leads to REMS approximately 50% of the time in both sexes, but the probability is lower for the females in the earliest hours of the light period.

The ratio between the number of REMS episodes that occurred without prior tRS compared to the total number of REMS episodes was also analyzed. These ratios were small, and the standard

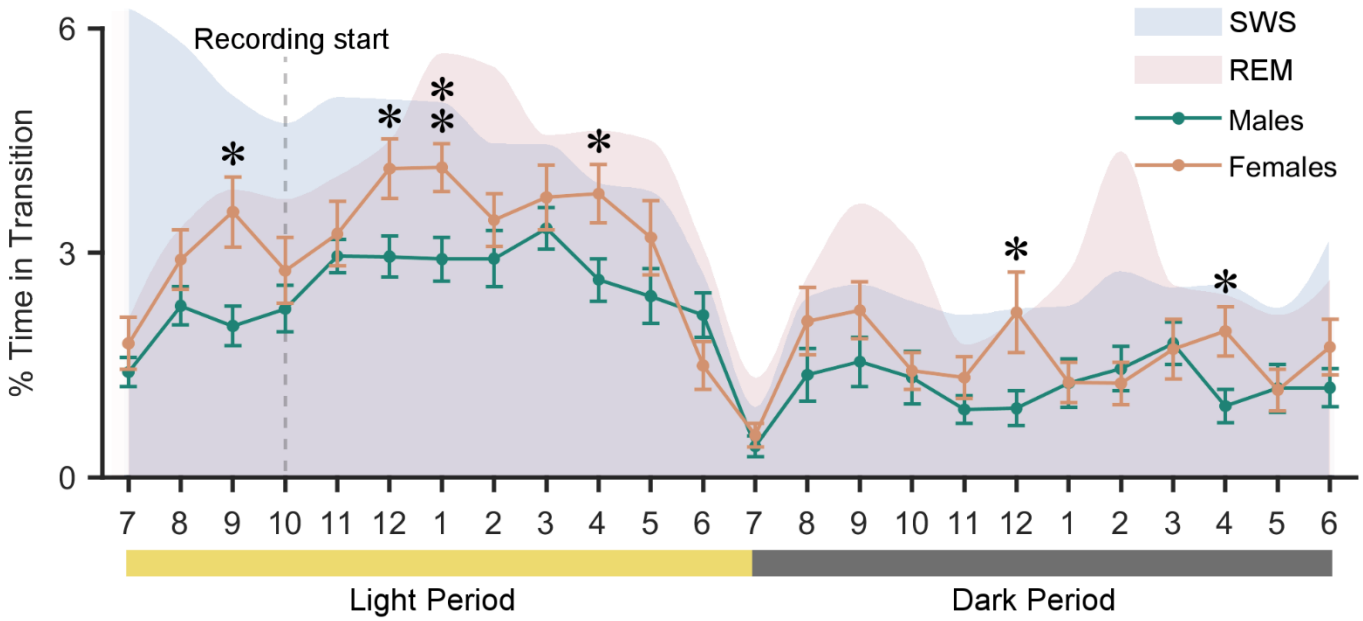

Figure 4.4. Sex Differences in the Hourly Expression of Transition Sleep.

The figure shows line plots of the average percentage of time spent in transition sleep for each hour of all four 24-hour recordings. The first half of the plot is the light phase, and the second half is the dark phase. Since recordings started at 10 AM, the first three hours shown are in fact the last three hours of the recordings; they are shown at the beginning for a better representation of the 24-hour sleep cycle. The combined average percentage of time spent in slow-wave sleep (SWS) and REM sleep (REM) are shaded in blue and red, respectively, behind the line plots. This allows for visualization of the relationships between slow-wave sleep, REM sleep, and transition sleep, but the shaded areas are not to scale with the y-axis. The slow-wave sleep shaded area is approximately 12.5 times larger than the axis scale, and the REM sleep shaded area is approximately 2 times larger than the axis scale. Note that females showed a higher percentage of time spent in transition sleep for most of the light phase, and a significantly higher percentage at 9 am, 12 pm, 1 pm, and 4 pm. Additionally, peak slow-wave sleep occurs very early in the morning, while peak REM sleep occurs in the afternoon. Also note that, in most cases, peaks in transition sleep tend to precede peaks in REM sleep, and transition sleep declines begin before large REM sleep declines. The latter half of the dark phase did not follow this trend. $* p < 0.05$, $** p < 0.01$.

deviations were relatively large ($mean_M$ = .067, SD_M = .065; $mean_F$ = .056, SD_F = .063). Therefore, it was unnecessary to run further statistical analyses to conclude that there were no differences between the sexes.

Sex Mediates the Relationships Between Sleep Stages

To better understand the relationships between all three sleep stages, linear regression modelling was performed, the results of which are presented in Table 4.1. Partial models of hour-to-hour variations in the percentage of time spent in each of the three stages of sleep were generated without controlling for the episode number and duration of the modelled sleep stage.

Interestingly, the tRS partial models were the most powerful, with SWS and REMS variables accounting for 59.6% of tRS variability in males and 58.7% of tRS variability in females. In general, SWS and REMS variables held positive relationships with time spent in tRS. For the males, the number of REMS episodes carried the most weight in predicting percent time in tRS (β=.401, $p < 0.001$). For the females, it was percent time spent in REMS that had the highest weight in the model (β = .525, $p < 0.001$). In both cases, it was REMS and not SWS that was most influential on tRS.

In the partial models of percent time spent in REMS, tRS variables held the strongest weights, although tRS and SWS collectively only accounted for 31.4% of the males' variability and 44.6% of the females' variability. The male variability was more related to the number of tRS episodes (β=.593, $p < 0.001$), and the female variability was more related to the percent of time spent in tRS (β = .594, $p < 0.001$), mirroring the relationships that were found in the tRS partial models. The SWS partial models put more weight on tRS than on REMS. In fact, REMS held no significant weight in either male or female SWS partial models, although there was a trend in the female model ($p = 0.057$). However, these models only accounted for 33.7% of the variability in percent of time spent in SWS for the males, and 31.7% for the females. Still, the evidence suggests that SWS and REMS variables do not hold much influence over each other when tRS is accounted for.

Full models were produced by controlling for the episode duration and frequency of the modelled sleep. In the full models of male SWS and REMS, the influences of other sleep stages disappeared entirely. Full models of female SWS showed significant effects of the number of tRS episodes (β = .076, $p < 0.01$) and percentage of time spent in REMS (β = .082, $p < 0.01$). Additionally, for the females, time spent in tRS still held the highest weight in the full model of REMS (β = .561, $p < 0.001$). Likewise, percent time spent in REMS still held the highest weight in the full model of tRS (β = .485, $p < 0.001$). For the males, the other sleep stages either had negative or non-significant influences on time spent in tRS.

The heatmaps in Figure 4.5 illustrate the sex differences in the relationships between sleep stages by mapping the β values of the different predictors for each model. For the males, the number of episodes held the most influence in most models, while it was the percentage of time that held the most influence for the females. In the full models, the males show a diagonal pattern in their heatmap, indicating each sleep stage was more related to its own parameters than to the other sleep stages. For the females, the heatmap does not hold this diagonal pattern across the tRS and REMS full models. Collectively, the data show that the females' tRS and REMS stages were heavily influenced by one another, while the males' sleep stages were more independently regulated.

Table 4.1. Linear Regression Models of Hourly Time Spent in Different Stages of Sleep

Males					
Model				**Coefficients**	
Dependent Variable	**Summary**	**Predictors**		**b**	**β**
% Time in Transition Sleep[a]	R^2 = .592 $F_{(6,545)}$ = 131.763 p < 0.001	REMS	% Time	.030*	.090
			Avg. Duration	3.135E-5**	.103
			Total #	.003***	.401
		SWS	% Time	.019**	.257
			Avg. Duration	-2.203E-5	-.080
			Total #	.000**	.187
% Time in Transition Sleep[b]	R^2 = .918 $F_{(8,543)}$ = 762.725 p < 0.001	tRS	Avg. Duration	.001***	.268
			Total #	.004***	.892
		REMS	% Time	.007	.020
			Avg. Duration	-1.410E-5**	-.047
			Total #	.000*	-.039
		SWS	% Time	.005	.065
			Avg. Duration	-1.998E-5*	-.072
			Total #	.000***	-.112
% Time in REM Sleep[a]	R^2 = .314 $F_{(6,545)}$ = 41.482 p < 0.001	tRS	% Time	-.241	-.080
			Avg. Duration	.001*	.177
			Total #	.007***	.593
		SWS	% Time	.021	.094
			Avg. Duration	-6.4003E-05	-.077
			Total #	-.001	-.098
% Time in REM Sleep[b]	R^2 = .487 $F_{(8,543)}$ = 64.352 p < 0.001	REMS	Avg. Duration	.000***	.334
			Total #	.009***	.422
		tRS	% Time	.375	.124
			Avg. Duration	-.001	-.090
			Total #	.001	.051
		SWS	% Time	.016	.073
			Avg. Duration	-4.847E-5	-.058
			Total #	.000	-.057
% Time in Slow-Wave Sleep[a]	R^2 = .337 $F_{(6,545)}$ = 46.078 p < 0.001	tRS	% Time	-3.171	-.231
			Avg. Duration	.011***	.307
			Total #	.035***	.630
		REMS	% Time	.045	.010
			Avg. Duration	-2.035E-5	-.005
			Total #	-.003	-.026
% Time in Slow-Wave Sleep[b]	R^2 = .887 $F_{(8,543)}$ = 533.895 p < 0.001	SWS	Avg. Duration	.003***	.754
			Total #	.023***	.660
		tRS	% Time	1.239	.090
			Avg. Duration	-7.110E-5	-.002

Table 4.1. Continued

Males					
Model				**Coefficients**	
Dependent Variable	**Summary**	**Predictors**		**b**	**β**
% Time in Slow-Wave Sleep[b]		tRS	Total #	-.001	-.025
		REMS	% Time	.073	.016
			Avg. Duration	-2.225E-5	-.005
			Total #	.000	.004
Females					
% Time in Transition Sleep[a]	$R^2 = .587$ $F_{(6,545)} = 129.341$ $p < 0.001$	REMS	% Time	.186***	.525
			Avg. Duration	-3.954E-5*	-.091
			Total #	.001**	.104
		SWS	% Time	.017**	.191
			Avg. Duration	-2.558E-5	-.079
			Total #	.001***	.256
% Time in Transition Sleep[b]	$R^2 = .672$ $F_{(8,543)} = 139.268$ $p < 0.001$	tRS	Avg. Duration	.001***	.342
			Total #	.001***	.158
		REMS	% Time	.172***	.485
			Avg. Duration	-8.961E-5***	-.206
			Total #	.000	-.018
		SWS	% Time	.008	.087
			Avg. Duration	-2.248E-5	-.070
			Total #	.001***	.213
% Time in REM Sleep[a]	$R^2 = .446$ $F_{(6,545)} = 73.149$ $p < 0.001$	tRS	% Time	1.674***	.594
			Avg. Duration	.001*	.093
			Total #	.000	-.030
		SWS	% Time	.061**	.239
			Avg. Duration	-9.276E-5	-.102
			Total #	-.001**	-.173
% Time in REM Sleep[b]	$R^2 = .621$ $F_{(8,543)} = 111.149$ $p < 0.001$	REMS	Avg. Duration	.001***	.476
			Total #	.003**	.120
		tRS	% Time	1.583***	.561
			Avg. Duration	-.001**	-.115
			Total #	-.001**	-.112
		SWS	% Time	.058**	.225
			Avg. Duration	-9.619E-5	-.106
			Total #	-.001***	-.190
% Time in Slow-Wave Sleep[a]	$R^2 = .317$ $F_{(6,545)} = 42.170$ $p < 0.001$	tRS	% Time	2.746***	.250
			Avg. Duration	.007***	.186
			Total #	.011***	.244
		REMS	% Time	.419	.108
			Avg. Duration	1.641E-5	.003

Table 4.1. Continued

Females					
Model				**Coefficients**	
Dependent Variable	**Summary**	**Predictors**		**b**	**β**
% Time in Slow-Wave Sleep[a]		REMS	Total #	-.007	-.082
% Time in Slow-Wave Sleep[b]	R^2 = .862 $F_{(8,543)}$ = 423.773 p < 0.001	SWS	Avg. Duration	.002***	.674
			Total #	.018***	.603
		tRS	% Time	.402	.037
			Avg. Duration	.001	.028
			Total #	.003**	.076
		REMS	% Time	.319**	.082
			Avg. Duration	.000	-.031
			Total #	-.003	-.032

a. Partial model that does not account for the number and duration of episodes of the modelled sleep stage.

b. Full model that does account for episode number and duration of the modelled sleep stage.

Gold highlighted cells indicate the predictor with the highest weight for each model.

* p < 0.05, ** p < 0.01, *** p < 0.001.

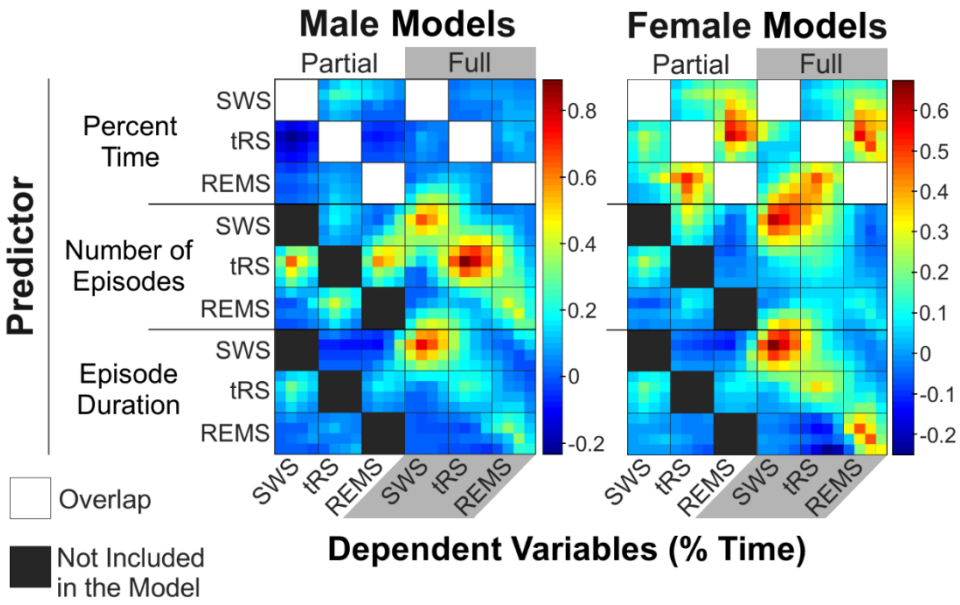

Figure 4.5. Sex Differences in the Relationships Between Stages of Sleep

The figure shows heatmaps illustrating the β values of each predictor in the partial and full models of the percentage of time spent in each stage of sleep. Red indicates a strong influence; blue indicates a weak influence. Note that, for the males, the red is concentrated in the middle of the heatmap, indicating that numbers of episodes were the strongest predictors of the percentage of time spent in each sleep stage. For the females, the red is concentrated at the top of the heatmap, indicated that it was percentages of time spent in other sleep stages that were the strongest predictors of the percentage of time spent in each sleep stage. Also note that the top-left to bottom-right diagonal pattern produced by predictor/model overlap is seen in the heatmap of the male full models, but for the females, that pattern is not seen in the transition sleep or REM sleep full model heatmaps. Abbr.: slow-wave sleep (SWS), transition sleep (tRS), REM sleep (REMS).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

This study illustrates that tRS is a distinct stage of sleep marked by cortical EEG signals containing low frequency delta waves in combination with spindle-like activity. During tRS, hippocampal EEG signals contain low frequency theta activity, as compared to REMS which is saturated with higher theta frequencies. Additionally, the results of this study indicate that tRS is moderated by sex. Female rats showed more frequent tRS episodes and a higher percentage of time spent in tRS in the light phase. However, female rats also showed shorter REMS durations compared to male rats. It is possible that the differences in REMS maintenance are related to the differences in transition sleep. Perhaps the shorter REMS durations in females result in a higher number of transition episodes, or vice-versa. Future investigations should focus on the relationship between tRS and REMS, and potential sex differences in this relationship.

Our hour-by-hour analyses suggest a circadian rhythm for tRS, since the averages of four different days show a distinct peak (Figure 4.4). Increases in transition sleep begin at noon and then taper off until about 4 pm. The REMS circadian rhythm shown in Figure 4.4 appears to be similar, but peaks begin and end an hour later, further illustrating the relationship between tRS and REMS. Interestingly, SWS predominated in the first part of the light phase, from 7 am at noon. Again, this provides evidence that SWS and REMS work in a cooperative process, with each performing a function that compliments the other. More investigations into the relationship between non-REM sleep and REM sleep are needed. To date, most sleep research has focused on non-REM and REM sleep as separate processes. Investigating tRS may provide the link needed to develop a cohesive understanding of sleep, both non-REM and REM.

For both males and females, the linear regression models of SWS and REMS showed a stronger association with tRS than with each other. Although this sounds intuitive, it provides evidence against claims that REM sleep is a response to SWS [21]. Instead, the data presented here indicate that there are mechanisms which promote tRS while in SWS, and mechanisms that promote REMS while in tRS. Elucidation of these mechanisms will not only provide a more complete understanding of the neurobiology of sleep but could also provide more insight into sleep dysregulations that involve alterations in the timing of REMS. Since debilitating psychiatric disorders such as depression, PTSD, and schizophrenia involve such alterations, this could provide novel ways of understanding and treating psychiatric illnesses.

Melanin-concentrating hormone (MCH) is a neuropeptide that might contribute to sex differences in sleep regulation [159]. MCH is most known for its involvement in regulating energy expenditure and motivated behaviors [160-162]. Populations of MCH neurons have been found in the incerta and lateral hypothalamic nuclei, with extensive projections to the cortex, midbrain, and brainstem (Figure A.4) [159]. There is also a population of MCH neurons in the paramedian pontine reticular formation, but its projection sites are currently unknown. Although MCH neurons were discovered in the 1980's, their role in regulating sleep was not reported until 2003 [163]. Interestingly, that report showed that intracerebroventricular injections of MCH induced significant increases in both SWS and REMS. After that initial finding, several other studies have further elucidated the role of MCH neurons in regulating sleep, and REMS in particular [159]. MCH neurons project to many areas

that regulate the sleep-wake cycle, including the pontine tegmentum, which has been shown to control the initiation of REMS.

A recent study using optogenetics showed that activation of MCH neurons in the lateral hypothalamus during non-REM sleep facilitated non-REM to REMS transitions, while activation during REMS stabilized and extended REMS episode durations [164]. Of note, this study only used male mice in their optogenetic sleep recordings and did not score a separate tRS stage. However, they did find that activation of MCH neurons induced a shift in the dominant theta peak towards a slower oscillation, which could be an indication that some of what they identified as REMS was actually be tRS. Combined with the results of this study, it is possible that males and females exhibit different patterns of MCH neuronal activity, with females having more activity during non-REM, resulting in more transitions, and males having more activity during REMS, resulting in longer REMS durations. A study involving optogenetic manipulation of MCH neurons in both sexes and the scoring of tRS would solidify the understanding of this potential mechanism.

In regard to sex differences in transition sleep, of particular interest is the fact that there are populations of MCH neurons found only in female rats (Figure A.4). These populations are found in the medial preoptic area (mPOA), the paraventricular nucleus (PVN), and the laterodorsal tegmentum (LDT) [159, 165]. Importantly, two of these areas have been implicated in sleep regulation – the mPOA has been shown to maintain sleep and inhibit sleep fragmentation, while the LDT has been implicated in the regulation of REMS [166, 167]. Additionally, the PVN is a critical regulator of the physiological responses to stress [168-171]. The sleep-related implications of these sex differences in MCH expression have not been investigated, but they provide a potential mechanism by which females have a different relationship between the stages of sleep, as well as a different relationship between hormones, stress and sleep disruption.

Having an intermediate sleep stage between SWS and REMS fits best with the idea that SWS and REMS have complimentary and cooperative, rather than competing functions. This idea is not new but still has not been fully investigated, and the proposed mechanisms have not been completely proven [18, 154]. That being said, if the optimal function of REMS depends on the brain having completed processes that are dependent on SWS as has been proposed, then there needs to be a mechanism by which the brain can determine that the SWS process has been sufficiently completed. Relying solely on the circadian timing of SWS and REMS (one occurring earlier and the other occurring later) would provide too many opportunities for suboptimal functioning. The transition sleep stage provides a period of time for the brain to determine whether or not it is "ready" for REMS. The fact that about 50% of tRS episodes do not result in REMS supports this theory. Since many neuropsychiatric illnesses are associated with dysregulated sleep, and this dysregulation often precedes other symptoms, it is possible that a dysregulation of transition sleep for extended periods of time leads to a disorganization of brain structure and function due to the plasticity-related functions of sleep. In short, without the ability to ensure enough SWS precedes REMS, the long-term potentiation induced by REMS will occur in inappropriate places due to an insufficient amount of depotentiation by slow-wave activity. Insufficient synaptic pruning has already been linked with numerous psychiatric disorders, and this potential sleep-related over-scaling of synaptic potentiation could have similar cognitive effects [172-175]. Investigating the changes in tRS that occur in models of neuropsychiatric disorders is an important step into investigating this possibility.

This study provides evidence that the mechanisms of tRS may be different in males and females, at least in rats. Our linear regression models suggest that each sleep stage in males is more independently regulated than in females, who seem to have sleep stages that are more reactive. This claim is supported by the fact that, in males, the full models of percent time spent in SWS and REMS did not contain significant influences from any other sleep stage; only tRS seemed to connect the two by having significant influences from each. However, in females, the full models still showed significant influences from the other sleep stages, and the percent time spent in tRS and REMS were more dependent on each other than on themselves or SWS. Additionally, in the partial models for males, it was the number of episodes that held the most weight in modeling percentages of time. In the females, it was percent time spent in other sleep stages that most influenced the percentage of time spent in each stage. In other words, for males, it is the act of shifting to or from a sleep stage that increases the percentage of time spent in that stage, while for females, the percentage of time spent in each sleep stage is more regulated by the percentage of time spent in the other sleep stages.

In light of other sex differences found in human sleep patterns, these results make sense. For example, by having sleep stages that are more interdependent, females have the opportunity to optimize their sleep, particularly in terms of the plasticity-related functions previously discussed. This is reflected both in the objectively better sleep quality found in women and their resilience to age-related changes in sleep. On the other hand, women would be more sensitive to changes in their sleep and small disruptions could quickly cascade into large disturbances. This correlates with the faster and more severe consequences of sleep loss shown in women compared to men, and their tendency to report subjectively poor sleep quality, which seems to contradict the objective findings. Additionally, the higher rates of depression and anxiety seen in women may be related to these sleep differences.

One major weakness of this study is the absence of estrous cycle data. Based on the sex differences seen in both humans and rodents, it is probable that differences in the stages of the estrous cycle would further illuminate sex differences in transition sleep, particularly since MCH expression varies between stages of the estrous cycle [159]. In rats, the length of the estrous cycle varies between four to six days [176]. Since recordings were taken every four days, each female was most likely sampled at more than one stage of her estrous cycle, so differences within the estrous cycle were most likely averaged out. While this is a strength when the estrous cycle isn't tracked, it does mean that there are potentially more differences between male and female rats than were found in this study. That is, there are potentially larger or different differences between males and females that could be seen at specific stages of the estrous cycle, but not in other stages. On that note, other studies in rats have found reduced amounts of REMS in females compared to males, which was not observed here, although females did have shorter average REMS durations [108, 145]. There have also been reports of lower amounts of sleep during the dark period in female rats, was not observed here at all [108]. It is possible that the lack of separation of the different stages of the estrous cycle prevented this study from detecting these sex differences. However, the fact that differences in tRS were still detected indicates that this sex difference is particularly strong; a fact which should be considered in future investigations into sex differences in sleep.

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APPENDIX

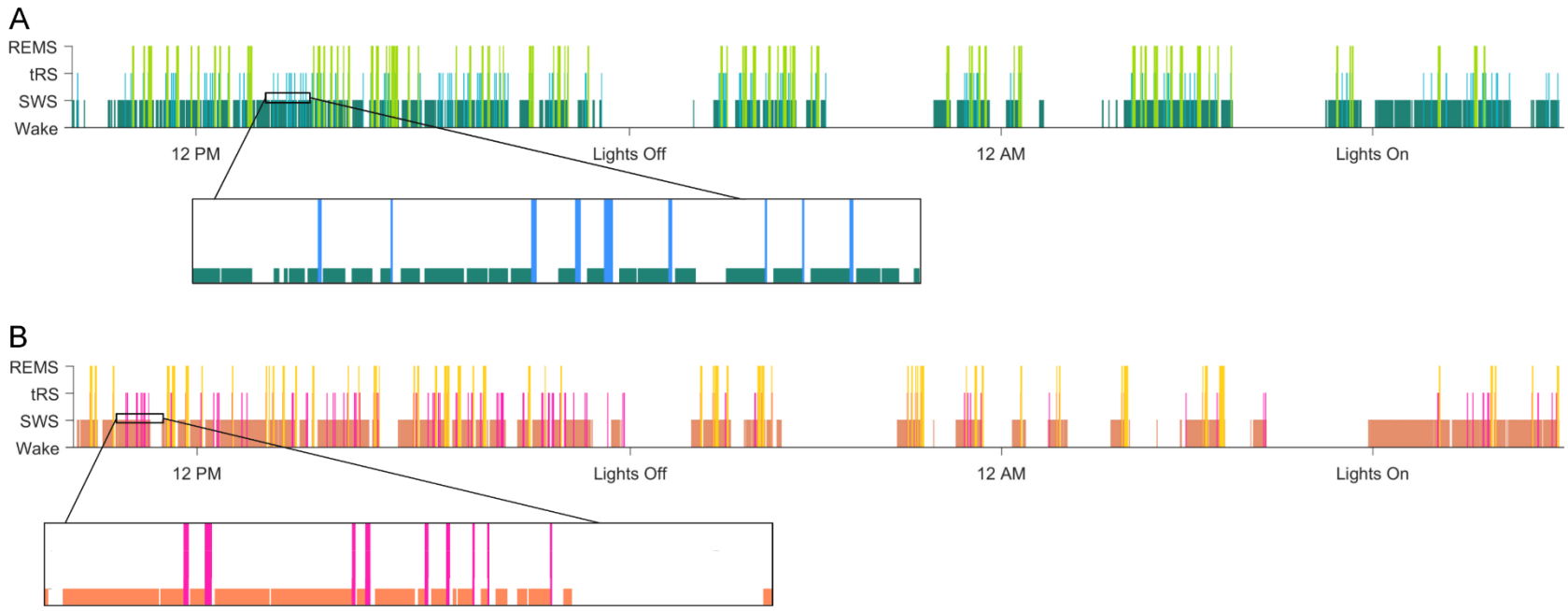

Figure A.1. Representative Hypnograms of a Male and Female Rat

The figure shows hypnograms of a 24-hour recording, starting and ending at 10 AM, for A) a male rat and B) a female rat. Zoomed segments are used to illustrate resolution. Lines of different height and color are used to represent episodes of the different stages of sleep and wakefulness. Segments of white space indicate wakefulness; segments reaching the first tick indicate slow-wave sleep (SWS); segments reaching the second tick indicate transition sleep (tRS); segments reaching the fourth tick, at the top, indicate REM sleep (REMS). Note that sleep predominates during the light period, at the beginning and end of the recordings, while wakefulness predominates during the dark period, in the middle of the recording, after the lights go off and before the come on. Also note that the female hypnogram shows much less sleep during the dark period, and the male hypnogram appears to have more REM sleep.

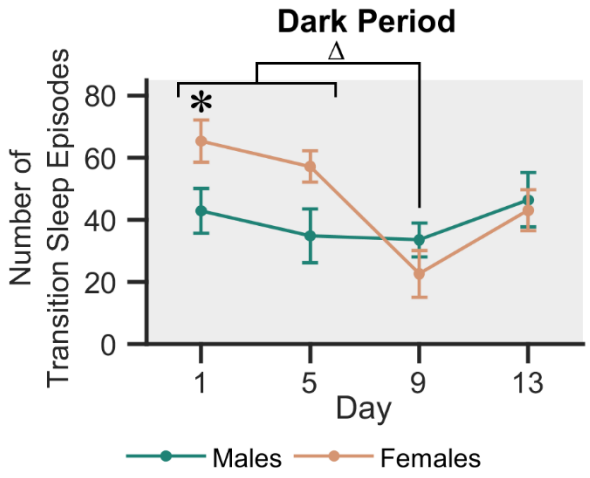

Figure A.2. Effects of Sex and Day on the Number of Transition Sleep Episodes

The figure shows line plots of the average number of transition sleep episodes for males and females in the dark phase of the four different 24-hour recordings. Both sexes showed a lower frequency of transition sleep on Day 9, compared to their respective frequencies on Days 1 and 5. Additionally, females showed a higher frequency of transition sleep compared to males on Days 1 and 5. The figure illustrates that transition sleep appears to be loosely regulated during the dark phase. $*$ compares sexes, $* p < 0.05$; Δ compares days, $\Delta p < 0.05$.

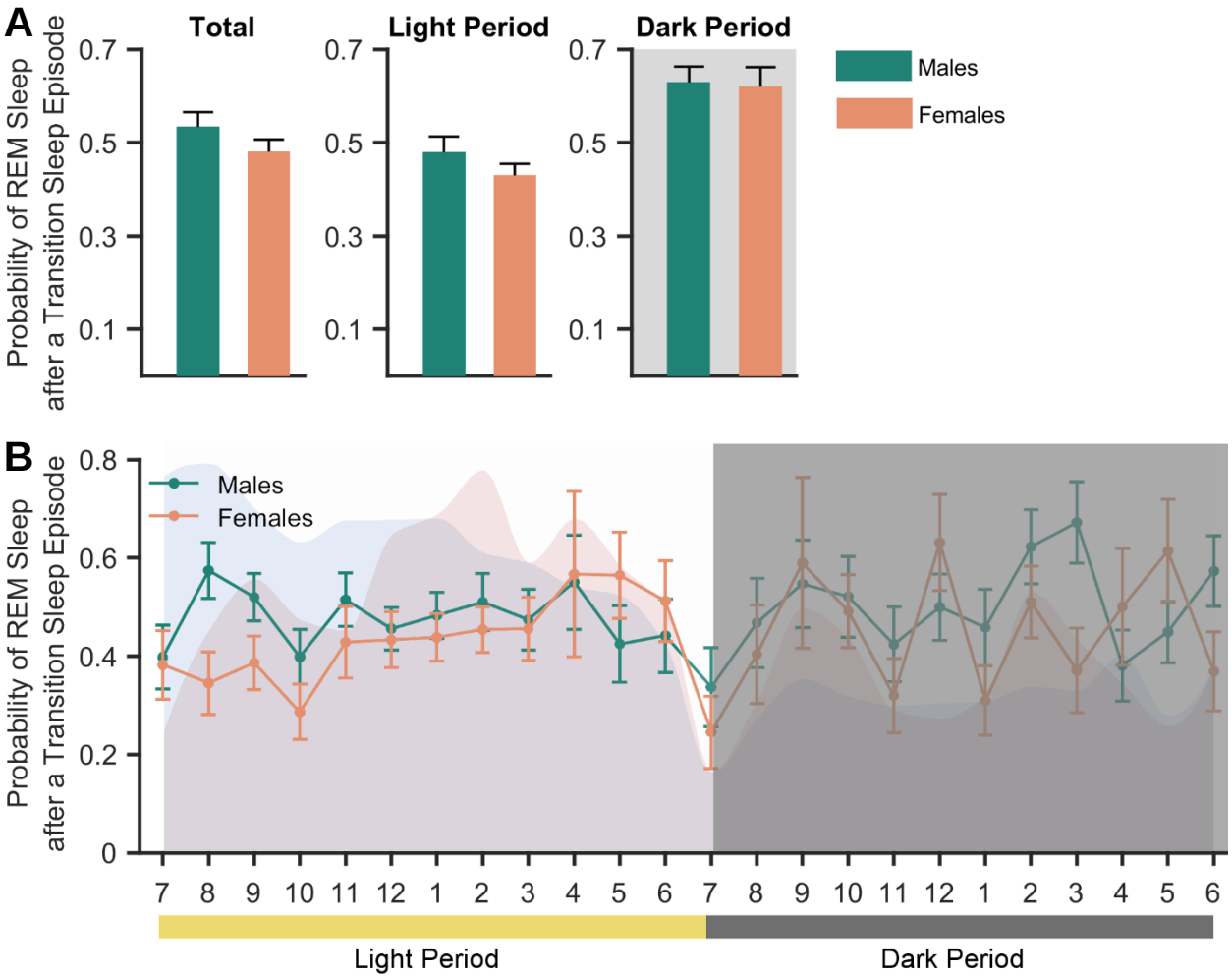

Figure A.3. Effects of Sex on the Probability that REM Sleep Will Follow Slow-Wave Sleep

The figure shows A) histograms representing the average probability of REM sleep following transition sleep across the total of all four 24-hour recordings, as well as in the light and dark periods of all the 24-hour recordings, and B) line plots of the average probability of REM sleep occurring after transition sleep in each hour of all four 24-hour recordings. Note that there were no sex differences when all hours were averaged, but when hours were individually inspected, there was a pattern in which males had a higher probability of transition leading to REM sleep in the beginning of the light period.

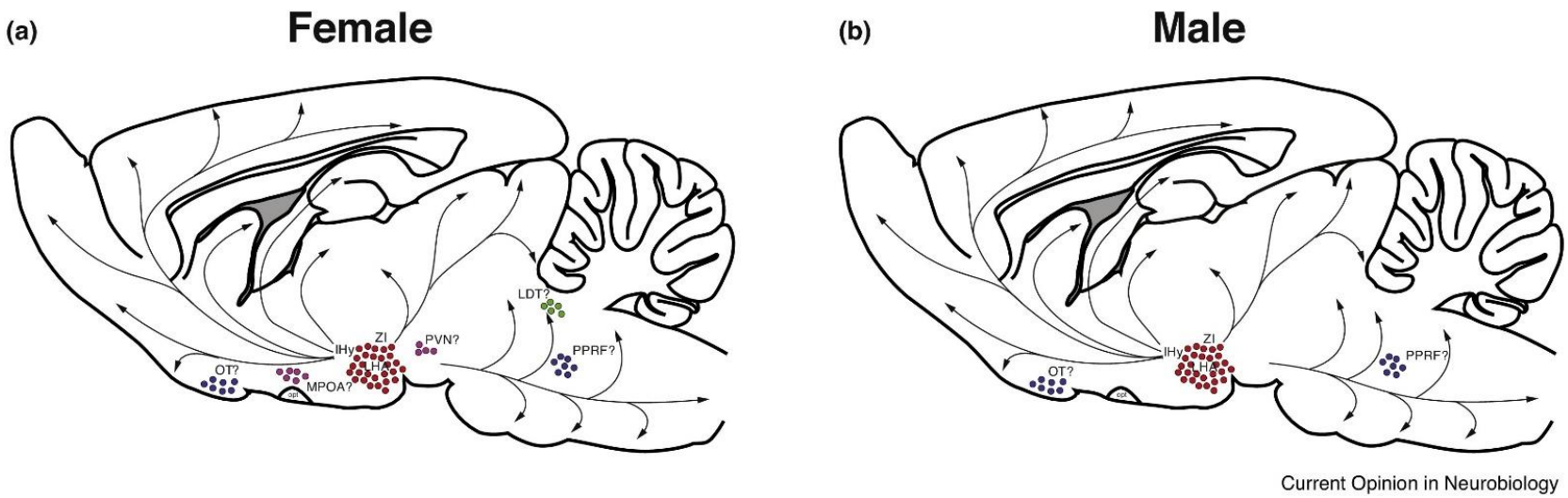

Figure A.4. Sexual Dimorphism in Populations of Melanin-Concentrating Hormone Neurons

MCH expressing neurons in the rat brain. Schematic sagittal representation of the (a) female and (b) male rat central nervous system illustrating the main areas where MCH neurons are found in both male and female: the zona incerta, lateral hypothalamic and the incerto hypothalamic areas (ZI, LHA, IHy; red) and their projections pattern. Extra-hypothalamic sites in both male and female: the olfactory tubercle and the paramedian pontine reticular formation (OT, PPRF; blue). The projections of these extra-hypothalamic sites are unknown. Novel sites of MCH expression only in female: the laterodorsal tegmental nucleus (LDT; green). During lactation, the MCH-expressing neurons can be found in the medial preoptic area and the paraventricular hypothalamic nucleus (MPOA, PVN; purple). The projections of these regions are still unknown.

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Table A.1. Sex Differences in the Percentage of Each Hour Spent in tRS

Hour	Mean Difference (female - male)	F(1,44)
10 am	.005	0.895
11 am	.003	0.374
12 pm	.012*	5.875
1 pm	.012**	7.963
2 pm	.005	1.000
3 pm	.004	0.648
4 pm	.012*	5.649
5 pm	.008	1.625
6 pm	-.007	2.404
7 pm	.001	0.485
8 pm	.007	1.565
9 pm	.007	1.874
10 pm	.001	0.041
11 pm	.004	1.630
12 am	.013*	4.805
1 am	7.826E-5	0.000
2 am	-.002	0.231
3 am	-.001	0.028
4 am	.010*	6.399
5 am	.000	0.003
6 am	.005	1.424
7 am	.004	0.928
8 am	.006	1.693
9 am	.015*	8.050

Shaded cells indicate hours during the dark period.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table A.2. Hourly Comparisons of the Percentage of Time Spent in tRS

Hour (I)	Hour (J)	Mean Difference (I-J)
10 am	11 am	.002
	12 pm	-.006
	1 pm	-.009*
	2 pm	-.006
	3 pm	-.004
	4 pm	-.006
	5 pm	-.002
	6 pm	.003
	7 pm	.019***
	8 pm	.017***
	9 pm	.008*
	10 pm	.012**
	11 pm	.014***
	12 am	.018***
	1 am	.014***
	2 am	.011**
	3 am	.014***
	4 am	.010**
	5 am	.016***
	6 am	.015***
	7 am	.012***
	8 am	.005
	9 am	-3.696E-05
11 am	12 pm	-.008**
	1 pm	-.011**
	2 pm	-.008*
	3 pm	-.006
	4 pm	-.008*
	5 pm	-.003
	6 pm	.001
	7 pm	.017***
	8 pm	.015***
	9 pm	.007
	10 pm	.010*
	11 pm	.013***
	12 am	.016***
11 am	1 am	.012**
	2 am	.010*
	3 am	.012**
	4 am	.008*
	5 am	.014***
	6 am	.013**
	7 am	.010*
	8 am	.003
	9 am	-.002
12 pm	1 pm	-.003
	2 pm	.000
	3 pm	.002
	4 pm	7.609E-05
	5 pm	.005
	6 pm	.009**
	7 pm	.025***
	8 pm	.024***
	9 pm	.015***
	10 pm	.018***
	11 pm	.021***
	12 am	.024***
	1 am	.020***
	2 am	.018***
	3 am	.020***
	4 am	.016***
	5 am	.023***
	6 am	.021***
	7 am	.018***
	8 am	.011***
	9 am	.006*
1 pm	2 pm	.003
	3 pm	.005
	4 pm	.003
	5 pm	.008*
	6 pm	.012***
	7 pm	.028***

Table A.2 Continued

Hour (I)	Hour (J)	Mean Difference (I-J)
1 pm	8 pm	.027***
	9 pm	.018***
	10 pm	.021***
	11 pm	.024***
	12 am	.027***
	1 am	.023***
	2 am	.021***
	3 am	.024***
	4 am	.019***
	5 am	.026***
	6 am	.024***
	7 am	.021***
	8 am	.014***
	9 am	.009**
2 pm	3 pm	.002
	4 pm	.000
	5 pm	.005
	6 pm	.009**
	7 pm	.025***
	8 pm	.023***
	9 pm	.014***
	10 pm	.018***
	11 pm	.021***
	12 am	.024***
	1 am	.020***
	2 am	.017***
	3 am	.020***
	4 am	.016***
	5 am	.022***
	6 am	.021***
	7 am	.018***
	8 am	.011***
	9 am	.006*
3 pm	4 pm	-.002
	5 pm	.003
	6 pm	.007*
3 pm	7 pm	.023***
	8 pm	.022***
	9 pm	.013***
	10 pm	.016***
	11 pm	.019***
	12 am	.022***
	1 am	.018***
	2 am	.016***
	3 am	.019***
	4 am	.014***
	5 am	.021***
	6 am	.019***
	7 am	.016***
	8 am	.009**
	9 am	.004
4 pm	5 pm	.005
	6 pm	.009**
	7 pm	.025***
	8 pm	.023***
	9 pm	.015***
	10 pm	.018***
	11 pm	.021***
	12 am	.024***
	1 am	.020***
	2 am	.018***
	3 am	.020***
	4 am	.016***
	5 am	.022***
	6 am	.021***
	7 am	.018***
	8 am	.011**
	9 am	.006
5 pm	6 pm	.004
	7 pm	.020***
	8 pm	.019***
	9 pm	.010**

Table A.2 Continued

Hour (I)	Hour (J)	Mean Difference (I-J)
5 pm	10 pm	.014***
	11 pm	.016***
	12 am	.019***
	1 am	.015***
	2 am	.013***
	3 am	.016***
	4 am	.011**
	5 am	.018***
	6 am	.016***
	7 am	.013***
	8 am	.007
	9 am	.001
6 pm	7 pm	.016***
	8 pm	.015***
	9 pm	.006
	10 pm	.009**
	11 pm	.012**
	12 am	.015***
	1 am	.011**
	2 am	.009**
	3 am	.011**
	4 am	.007*
	5 am	.013***
	6 am	.012**
	7 am	.009**
	8 am	.002
	9 am	-.003
7 pm	8 pm	-.001
	9 pm	-.010**
	10 pm	-.007*
	11 pm	-.004
	12 am	-.001
	1 am	-.005
	2 am	-.007**
	3 am	-.005
	4 am	-.009**
	5 am	-.002
	6 am	-.004
	7 am	-.007*
	8 am	-.014***
	9 am	-.019***
8 pm	9 pm	-.009**
	10 pm	-.005
	11 pm	-.003
	12 am	.001
	1 am	-.003
	2 am	-.006
	3 am	-.003
	4 am	-.007*
	5 am	-.001
	6 am	-.002
	7 am	-.005
	8 am	-.012***
	9 am	-.017***
9 pm	10 pm	.004
	11 pm	.006*
	12 am	.009**
	1 am	.005
	2 am	.003
	3 am	.006
	4 am	.001
	5 am	.008*
	6 am	.007
	7 am	.003
	8 am	-.003
	9 am	-.008**
10 pm	11 pm	.002
	12 am	.006*
	1 am	.002
	2 am	-.001
	3 am	.002
	4 am	-.002

Table A.2. Continued

Hour (I)	Hour (J)	Mean Difference (I-J)
10 pm	5 am	.004
	6 am	.003
	7 am	.000
	8 am	-.007*
	9 am	-.012**
11 pm	12 am	.003
	1 am	-.001
	2 am	-.003
	3 am	.000
	4 am	-.005
	5 am	.002
	6 am	.000
	7 am	-.003
	8 am	-.009**
	9 am	-.015***
12 pm	1 am	-.004
	2 am	-.006**
	3 am	-.004
	4 am	-.008*
	5 am	-.002
	6 am	-.003
	7 am	-.006*
	8 am	-.013***
	9 am	-.018***
1 am	2 am	-.002
	3 am	.000
	4 am	-.004
	5 am	.002
	6 am	.001
	7 am	-.002
1 am	8 am	-.009*
	9 am	-.014***
2 am	3 am	.003
	4 am	-.002
	5 am	.005*
	6 am	.004
	7 am	.000
	8 am	-.006
	9 am	-.011**
3 am	4 am	-.004
	5 am	.002
	6 am	.001
	7 am	-.002
	8 am	-.009**
	9 am	-.014***
4 am	5 am	.006*
	6 am	.005
	7 am	.002
	8 am	-.005
	9 am	-.010***
5 am	6 am	-.001
	7 am	-.004*
	8 am	-.011***
	9 am	-.016***
6 am	7 am	-.003
	8 am	-.010**
	9 am	-.015***
7 am	8 am	-.007**
	9 am	-.012***
8 am	9 am	-.005*

Shaded cells indicate hours during the dark period.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

VITA

Abigail Barnes was born in Princeton, New Jersey, on November 28, 1990, to her parents Boyd and Elaine Barnes. In 1992, her parents moved her and her older brother Adam to Nashville, Tennessee, so that her father could attend graduate school at Vanderbilt University. In the summer of 2008, Abigail moved back to New Jersey with her father and completed her primary education at Hightstown High School. In the fall of 2010, Abigail headed back to Tennessee for college, attending the University of Tennessee, Knoxville. There, she was introduced to biological psychology. Abigail worked as an undergraduate assistant in two different research laboratories before graduating in December 2014. In March of 2015, Abigail attained a position as a Research Associate in a new Sleep and Cognitive Neuroscience research laboratory in the University of Tennessee Medical Center. In August of 2016, Abigail was admitted into the Experimental Psychology Graduate Program at the University of Tennessee, Knoxville. Abigail continued to work in the Sleep and Cognitive Neuroscience Laboratory and graduated with a Master of Arts degree in Psychology in May 2019, at which point she was looking forward to beginning work as a science journalist.