Circadian and homeostatic components of sleep across sex and
development in the diurnal rodent, *Octodon degus*

by

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Dedication

To my mom, dad, family, and friends

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Abstract

      This dissertation examined the impact of sex and development on sleep in the diurnal rodent, *Octodon degus*. All experiments utilized electrophysiological measures to quantify sleep patterns, brain temperature and locomotor activity (via electroencephalography, thermistor probes, and infrared motion detectors). The descriptive study revealed the diurnal non rapid eye movement sleep (NREMS) patterns of degus and the low levels of rapid eye movement sleep (REMS), with females showing significantly more NREMS amount and consolidation and less REMS compared to males. Sleep intensity (measured by NREMS delta wave activity) was sexually dimorphic, with males demonstrating higher relative levels during the light phase and females exhibiting increases during the dark phase. Circadian gating of sleep was particularly powerful, with both sexes displaying heightened activity around the light-dark transitions. The second set of experiments aimed to elucidate the homeostatic and circadian components of sleep utilizing the 6h sleep deprivation paradigm. Sleep deprivation increased homeostatic drive within both sexes and was mediated by circadian phase. Compensatory mechanisms for sleep recovery differed between sexes, with males demonstrating transient increases in NREMS amount and consolidation while females displayed transitory increases in NREMS consolidation and prolonged elevation of sleep intensity. Lastly, the final

experiments aimed to investigate the two components of sleep across development within male degus. While early pubertal degus did not demonstrate a strong preference for diurnal sleep, the rhythmicity of sleep intensity was consistent with previous studies of diurnal mammals. Late pubertal degus demonstrated circadian variation of NREMS and REMS, suggesting there may be a critical window of hormonal influence on sleep within this diurnal rodent. Both age groups displayed significantly increased NREMS parameters in response to a 6h deprivation during the dark phase, yet the circadian component of sleep was dampened during light transition periods, contrasting adult degus patterns. Together, these data highlight *Octodon degus* as a good diurnal rodent model for investigating the circadian and homeostatic mechanisms of sleep, as well as the interaction of these opponent processes under baseline conditions and following physiological disruption. Furthermore, the slowly developing degu presents a unique opportunity to examine these components in a small diurnal mammal.

Chapter I

Introduction

The Impact of Insufficient Sleep

Over the past 100 years, the amount of time that Americans sleep has decreased by approximately 20 percent (NCSDR, 1994). Furthermore, 1 out of every 5 workers in industrialized countries perform shift work, requiring them to work at night and attempt to sleep during the daytime (AASM, 2005). These reversed sleep cycles lead to circadian desynchrony, which leads to sleep disruption (Torsvall et al., 1989; Akerstedt, 2003). Additionally, a large part of the population experiences "social jetlag" (voluntarily pushing back sleep bedtimes, which ultimately leads to chronic sleep deprivation/curtailment), due to the limitless access to television and internet (Wittmann et al., 2006).   In total, it is estimated that 50-70 million Americans experience chronically disturbed sleep (NHLBI, 2003), impairing daily functioning and increasing the risk of adverse of health outcomes. One of the most prevalent chronic sleep disturbances is insomnia, which is characterized by an inability to fall asleep/stay asleep and is

observed in roughly 10 percent of the American population (Ford & Kamerow, 1989; Ancoli-Israel & Roth, 1999). Moreover, obstructive sleep apnea is a disorder marked by brief periods of recurrent cessation of breathing caused by airway obstruction and accounts for approximately 3 to 4 million individuals suffering from disrupted sleep (Young et al., 1993). Furthermore, restless legs syndrome and periodic limb movement disorder (characterized by nocturnal limb movements) affect roughly 5 percent of the general population (Lavigne & Montplaisir, 1994; NSF, 2000; Montplaisir et al., 2005). There are a myriad of situations that can result in lost/disrupted sleep, including the aforementioned ones. However, I will focus on data generated by sleep loss from acute or chronic sleep deprivation, as opposed to the aforementioned cases where underlying physiological disorders may directly impact sleep in addition to the consequences of sleep disruption.

The deleterious consequences of sleep loss and sleep-related disorders on public health are staggering (IOM, 2006). Many disasters (such as the nuclear reactor meltdowns at Chernobyl and Three Mile Island and the Exxon Valdez oil tragedy) instigated by humans have been largely attributed to sleep loss induced performance failures, with vast negative consequences to both the health of the human population and the environment (NCSDR, 1994; United States Senate Committee on Energy and National Resources, 1986; USNRC, 1987; Dinges et al.,1989). Individuals with chronic sleep loss are at increased risk of injury compared to their healthy counterparts (Lyznicki et al., 1998), with approximately 110,000 sleep-related injuries and 5,000 fatalities annually in

motor vehicle accidents attributed to sleep-impaired individuals (CNTS, 1996).

Moreover, sleep loss and conditions causing sleep disruption have large negative

economic consequences, with billions of dollars every year sacrificed to lost

productivity, work absenteeism, doctor visits, and prescription and over the

counter medications (NCSDR, 1994; Ricci et al., 2007). The aforementioned

data clearly demonstrates that sleep loss and sleep-related disorders are a

substantial problem within the general population that continues to be

inadequately addressed among the public health, health care and biomedical

research fields. The remainder of this chapter will briefly summarize knowledge

regarding (1) basic background of sleep architecture and physiology; (2) the

relationship between sleep loss and adverse health outcomes; (3) the basic

patterns of sleep across the lifespan, between sexes and how these factors

mediate the impact of sleep loss; (4) the gap in research investigating these

factors; and (5) how the animal model I propose for study can elucidate how

these factors impact sleep loss and its mechanism of recovery.

Sleep Architecture and Physiology

Sleep is a reversible state that is nearly ubiquitous across animal species.

This "resting" behavior is expressed differently across species, but is relatively

homologous in its characteristics and is vital for survival of most organisms.

While behavioral sleep has long been the center of inquiry and speculation,

scientific research investigating sleep utilizing electroencephalography (EEG)

only began during the twentieth century. Early work examined the architecture of

various states of sleep and wakefulness in the brain, along with its associated physiological correlates.

Sleep architecture is the basic organization of normal sleep and involves the quantification of electrophysiological characteristics to identify and characterize distinct stages (Jouvet, 1967). Sleep is divided into two major stages and these stages are mediated by numerous factors, including circadian rhythmicity, hormone levels, and sleep regulatory substances such as adenosine and melatonin among others (Dijk & Czeisler, 1995; Blanco-Centurion et al., 2006). The first sleep stage, non-rapid eye movement sleep (NREMS), is characterized by low muscle tone and low frequency-high amplitude waveforms (Kas & Edgar, 1998; Figure 1.1). The delta frequency band (0.5-4.5Hz) is a commonly used hallmark of NREMS. It is commonly used to quantify sleep intensity because previous electrophysiological studies have demonstrated a higher delta frequency band during NREMS is an indicator of sleep depth, frequently referred to as delta power. Human NREMS is further divided into sub-stages (Stage 1, Stage 2, and Stage 3) which have unique electrophysiological characteristics (Rechtshaffen & Kales, 1968), yet for the purpose of this introduction, NREMS will be discussed as a general sleep stage. The second major sleep stage is rapid eye movement (REMS), which is associated with low amplitude and mixed frequency waves, accompanied by rapid eye movements and muscle atonia (Rechtshaffen & Kales, 1968). The theta frequency band (4.5-7.5 Hz) is predominant during this sleep stage.

In order to better understand the fundamental complexity of sleep, a comprehensive model of sleep-wake regulation was theorized by Borbely (1982) known as the 2-process model (Figure 1.2). Borbely hypothesized sleep and wakefulness were regulated by the interaction of two systems (the homeostatic process "Process S" and the circadian process "Process C"). Process S, commonly referred to as homeostatic sleep drive, serves as a marker for the increase in sleep "need" due to extended wakefulness within animals, peaks during the early night, and dissipates across the predominant sleep phase (Borbely, 1982; Koorengevel et al., 2002; Gillette & Abbott, 2005). Sleep homeostasis serves to inform the organism of the amount of prior wakefulness. Conversely, the circadian component consists of biologically driven behavioral states that modulate across the day, alternately promoting arousal and sleepiness levels depending on phase (Mistlberger, 2005). Process C informs the organism of the time of day. The circadian process is controlled by the suprachiasmatic nucleus (SCN), which is located within the anterior hypothalamus and mediates sleep in concert with the homeostatic drive, creating a "trade-off" between the two processes (Mistlberger, 2005). The interactions between duration of prior wakefulness and time of day largely determine when sleep onset occurs.

Figure 1.3 illustrates the anatomical framework that generates the sleep and waking systems within the brain. Sleep is regulated by nuclei containing neurons that inhibit the arousal systems, which enables the initiation and maintainence of sleep (Figure 1.3A). The hypothalamus, specifically the ventral

lateral preoptic area (VLPO), contains many of these inhibitory neurons and the loss of these neurons has resulted in profound and long lasting insomnia (Gaus et al., 2002). Pontine neurons demonstrate an integral role in REMS generation and exchanging sleep states during the rest cycle (Saper et al., 2005), while inputs from other areas (i.e. the lower brainstem and forebrain) additionally influence sleep.

Wakefulness is generated by an arousal system originating from the brainstem (Saper et al., 2005). Two main brainstem pathways influence the arousal system by providing the majority of sensory input. The cholinergic neurons (from the upper pons) transmit sensory information via the thalamus to the cortex. Additionally, the upper brainstem nuclei containing monoamine neurotransmitters (i.e. locus coerulus, raphe nuclei, etc.) send sensory information via the hypothalamus and the basal forebrain to the cerebral cortex (Saper et al., 2005). The wakefulness and sleep generation systems inhibit one another to regulate the sleep-wake cycle.

The most important mammalian sleep research findings (such as the neuroanatomy of sleep regulation, rapid eye movement sleep, the 2 process model of sleep regulation among many others) have occurred within the last 70 years (Aserinsky & Kleitman, 1953; Jouvet, 1967; Borbely, 1982). Despite the revolutionary findings that have occurred within the past century, we have yet to provide conclusive evidence of why sleep is a necessary state for survival. The function of sleep has long been a topic of debate within the research community. While research has not found a sole primary function for sleep, increasingly the

research community has recognized the vital role sleep plays in a host of physiological, endocrine and psychological processes (Van Cauter et al., 2000; Sharma & Mazmanian, 2003; Tsuno et al., 2005; Gais et al., 2007).

A Role for Sleep in Health

Sleep loss is associated with many deleterious health effects (IOM Report, 2006). Sleep loss commonly refers to shorter than average sleep duration (<7 hours per night) and is connected with excessive daytime sleepiness, depressed mood and poor cognitive performance (Dinges, 2005). At least one-fifth of the general U.S. adult population reports getting insufficient sleep (Liu et al., 2000; Strine & Chapman, 2005), with a significantly increased proportion who sleep less than 6 hours compared to two decades ago (CDC, 2005). Conversely, over forty years ago, adults received 7.7 hours of sleep per night via self-report (Tune, 1968). Human studies have provided evidence that chronic sleep loss/deprivation strongly links with negative health outcomes such as obesity, diabetes/glucose intolerance, cardiovascular disease (CVD) and hypertension, cognitive impairments and psychiatric illnesses (Spiegel et al. ,1999; Ayas et al., 2003a; Ayas et al., 2003b; Fredriksen et al., 2004; Hasler et al., 2004; Liu, 2004; Taheri et al., 2004; Dinges, 2005; Gottlieb et al., 2005; Hasler et al., 2005; Strine & Chapman, 2005; Yaggi et al., 2005; Gangwisch et al., 2006). While many studies have examined the transient effects of acute (or total) sleep deprivation, work examining the consequences of chronic (partial) sleep loss is particularly

relevant for understanding the relationship between sleep and negative health outcomes.

Research examining the relationship between sleep loss and metabolic dysfunction has produced particularly robust results. Metabolic dysfunction is thought to be the primary cause for the obesity epidemic, with many studies demonstrating its association with sleep loss by measuring body mass index (BMI) (Taheri et al., 2004; IOM, 2006). Individuals who slept less than 6 hours were seven times more likely to have a higher BMI, even after controlling for possible confounding factors (Hasler et al., 2004; Taheri et al., 2004). Additionally, Taheri and colleagues (2004) investigated the physiological mechanism of metabolic dysfunction and found insufficient sleep increased hunger by measuring two appetite-related hormones (leptin and ghrelin). Restricted sleep was associated with lower levels of leptin (an appetite-suppressant) and higher levels of an appetite-stimulating peptide called ghrelin (Spiegel et al., 2004; Taheri et al., 2004). Furthermore, sleep curtailment was associated with a two-fold increase incidence of diabetes (Ayas et al., 2003b; Gottlieb et al., 2005). However, outside of metabolic dysfunction, many other adverse health outcomes are associated with sleep loss.

Sleep loss is linked with increased general mortality rates (Kripke et al., 2002; Patel et al., 2004), especially those associated with cardiovascular disease. Increased incidence in heart attacks occur with sleep deficiency (Liu & Tanaka, 2002; Ayas et al., 2003c; Yaggi et al., 2005). In one longitudinal study among nurses, no participants had heart disease during initial screening, yet ten

years later short-sleepers had an 39% increase in CVD risk even after controlling

for night shift work (Ayas et al., 2003b). Experimental data demonstrated acute

sleep deprivation leads to increased blood pressure in young healthy males

(Tochikubo et al., 1996; Meier-Ewert et al., 2004) and chronic sleep loss is linked

to hypertension (Gangwisch et al., 2006). In addition, sleep loss (acute and

chronic) has been associated with suppressed immune function in both human

and animal studies, illustrating the critical role sleep plays in mediating physical

wellbeing (Everson, 1993; Irwin et al., 1994; Irwin et al., 1996; Everson & Toth,

2000).

Additionally, sleep mediates mental well-being and sleep loss is

associated with many cognitive deficits and psychological disturbances when

sleep loss occurs (Van Dongen et al., 2003; Hasler et al., 2005; Strine &

Chapman, 2005). Insufficient sleep is linked with numerous negative

neurobehavioral effects, including unstable sustained attention with increased

errors, declining working memory short term recall, impaired learning of cognitive

tasks, and task performance deteriorating in a cumulative manner (Belenky et al.,

2003; Van Dongen et al., 2003; Lockley et al., 2004; Durmer & Dinges, 2005).

Human errors in judgment and decision making, as well as increased risk taking

behavior have been associated with sleep curtailment as well (Lockley et al.,

2004; Roehrs & Roth, 2004). Surprisingly, most participants were oblivious of

their performance decline when asked to evaluate themselves (Belenky et al.,

2003). Moreover, many psychological disturbances are prevalent in addition to

cognitive impairments.

For instance, insufficient sleep is correlated with increased emotional reactivity, as well as higher levels of disturbed mood, depressive symptoms, behavior problems, anxiety, substance use and abuse, and suicidal ideation and attempts in adults and children (Morrison et al., 1992; Wolfson & Carskadon, 1998; Liu, 2004; Hasler et al., 2005; Strine & Chapman, 2005). In children, sleep loss was associated with more depressive symptoms and decreased self-esteem over time beginning as early as middle school (Fredriksen et al., 2004), demonstrating the integral role sleep plays in the regulation of emotional affect and self-image across the lifespan. Furthermore, insomnia, which leads to inadequate amounts of sleep, has been associated with depression, anxiety and other psychiatric disorders (Breslau et al., 1996; Cole & Dendukuri, 2003; Ohayon & Roth, 2003). Research suggests sleep and mood disorders may have overlapping neurocircuitry (Jones, 2005; Nofzinger, 2005), potentially signifying that understanding the underlying anatomical dysregulation associated with inadequate and disrupted sleep may provide invaluable insight into psychiatric disturbances as well.

Although these studies provide compelling evidence demonstrating the association of sleep loss with adverse physical and mental health outcomes, most cannot determine causality or etiology due to their correlational or observational design. Thus, animal studies may help elucidate whether sleep loss *causes* these health outcomes or are simply an associated symptom. There is a critical need for basic research to thoroughly examine the clinical findings of the aforementioned studies and, ultimately, to understand the underlying

mechanisms of these sleep-related health disturbances. Animal studies may

provide insight into the mechanisms and underlying neurobiological framework

driving these negative outcomes.

Factors That Impact Sleep

While sleep research has increasingly focused on the impact of sleep loss

and its associated health effects, very little attention has been devoted to

investigating how these relationships may differ between sexes or with aging.

Large changes in sleep occur across the lifespan, particularly during infancy

through young adulthood, when rapid brain development and re-organization

takes place (Roenneberg et al., 2004; Feinberg et al., 1990; Figure 1.4). Sleep

rapidly changes across human infancy, with REMS (or "active sleep") accounting

for more than three-fourths of total sleep time (Frank & Heller, 1997). The

amount of REMS declines across development and reaches stable levels in early

adulthood, indicating a transient need for high levels of this behavioral state

(Ehlers & Kupfer, 1997). It has been hypothesized the predominance of REMS is

linked to synaptic development and plasticity, and data supports this theory

(Frank & Heller, 1997; Frank & Heller, 2003). Because cortical density also

declines with aging (Feinberg et al., 1990; Feinberg et al., 2006), researchers

associated this decrease in synaptic density with REMS, highlighting studies that

have demonstrated curtailment of REMS decreases synaptic connections and

facilitates learning and memory deficits (Harrison & Horne, 2000; Alhaider et al.,

2010). The impact of sleep loss on sleep amount and depth diminishes with advancing aging (IOM Report, 2006; Munch et al., 2004; Cajochen et al., 2006).

Although REMS is the dominant sleep state during infancy, NREMS becomes the prominent sleep stage during early childhood and adolescence. Delta power, a primary indicator of sleep intensity in NREMS, is elevated during adolescence and subsequently decreases with age (Feinberg et al., 1990; Feinberg et al., 2006). Moreover, the decline of delta power closely follows the decline of cortical density, suggesting NREMS, specifically intensity, may be correlated to synaptic pruning that is characteristic of adolescence (Feinberg et al., 1990). Sleep demonstrates more prominent sexually dimorphic patterns during adolescence, with females experiencing an earlier onset of changes, while males demonstrate delayed, yet more dramatic changes in sleep architecture and sleep intensity (Roenneberg et al., 2004; Hagenauer et al., 2009). Sexually differentiated sleep patterns persist into early adulthood and increase with advancing age (Bliwise, 1993; Ehlers & Kupfer, 1997).

Previous studies examining baseline sleep in both sexes demonstrated that many differences in the sleep architecture, intensity, timing and consolidation are sexually dimorphic (Dijk et al., 1989a; Armitage & Hoffmann, 2001). Females demonstrate increased NREMS, delta power and sleep consolidation in both human and animal studies (Wever, 1984; Mourtazaev et al., 1995; Driver et al., 1996). Conversely, males have demonstrated increased amounts of REMS compared with their female counterparts (Yamaoka, 1980; Fang & Fishbein, 1996). These sexually dimorphic differences appear to increase across the

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lifespan in humans, as young females and males demonstrate very subtle differences in sleep parameters (delta power not withstanding) which become more robust with age (Ehlers & Kupfer, 1989; Bliwise, 1993; Ehlers & Kupfer, 1997; Manber & Armitage, 1999; Koehl et al., 2006).

During baseline sleep recordings, women exhibit higher delta power amplitude than men (Armitage, 1995; Armitage et al., 1997), yet there was no sex difference in the accumulation or dissipation of delta power. The results highlight the complexity of comparing sleep between the sexes and suggest there may be subtle sex differences in homeostatic sleep regulation under baseline conditions (Armitage et al., 2000a, 2000b; Yamaoka, 1980; Fang & Fishbein, 1996). Follow up experiments provided evidence that females demonstrate a greater enhancement of delta power in response to sleep deprivation than males (Armitage & Hoffmann, 2001).

Further analysis, utilizing exponential regression to examine homeostatic regulation, supported previous evidence of a sex-mediated difference in sleep regulation and associated homeostatic processes (Armitage & Hoffmann, 2001). Sleep deprived women demonstrated a higher delta power asymptote and a faster rate of decay compared to their baseline sleep recordings. Sleep deprivation studies utilizing men revealed delta power during recovery sleep was directly related to baseline levels (Driver et al., 1996), suggesting males may encounter a ceiling effect mediated by previous levels. Females failed to demonstrate a ceiling effect of delta power recovery levels related to baseline levels. Additionally, following a deprivation protocol, males demonstrated normal

sleep patterns the following day, while females failed to return to baseline sleep levels, suggesting females demonstrate a longer response to sleep deprivation and may have a different compensatory mechanism to recover lost sleep (Corsi-Cabrera et al., 2003). However, findings have been inconsistent for females of all species, possibly due to the effects of fluctuating gonadal hormone levels on sleep regulation.

Due to the complexity of investigating female sleep, they have largely been ignored in both clinical and animal studies. Recently, there has been a resurgence of studies examining sex differences in the sleep research field (Andersen & Tufik, 2008; Deurveilher et al., 2009; Shechter & Boivin, 2010), which may be particularly important for translational research. Specifically, the prevalence of particular sleep disturbances and the incidence of associated health issues differ between sexes and will likely require different interventions (Edinger & Means, 2005; IOM, 2006). Moreover, sleep loss appears to more detrimental for women compared to their male counterparts. Research investigating the sexually dimorphic characteristics of sleep under varying conditions may prove particularly useful in translating research findings into more individualized and effective treatment for improving sleep quality. Furthermore, more developmental sleep studies may elucidate how sleep regulation evolves with age and may illuminate mechanisms that mediate sex differences in sleep-wake regulation.

Many of the developmental and sex difference animal sleep studies have utilized nocturnal animals. There are several limitations to exclusive use of

nocturnal rodents. Firstly, developmental studies examining sleep during puberty

remain a challenge due to the rapid rate of maturation within rats and mice.

Additionally, investigating the sleep of intact females is difficult due to the rapid

estrous cycling (~4-7 days) that occurs (Marrone et al., 1976; Kent et al., 1991).

Furthermore, sleep studies examining sex differences of nocturnal rodents have

produced mixed findings depending on the species (Yamaoka, 1980; Fang &

Fishbein, 1996). While nocturnal rodents share many characteristics with

humans, sleep regulation may exhibit important differences based on species

chronotype. Further sleep research that utilizes diverse species is essential,

particularly diurnal animals that may share more similarities with human sleep,

thereby providing additional translational value.

Degus: A Diurnal Animal Model for Human Sleep?

Octodon degus (degu) may serve as a good diurnal model for human sleep.

Degus are a long-lived, diurnal, and social species (Goel &Lee, 1995; Lee, 2004)

with a relatively long developmental maturation period for a rodent species. The

average degu lifespan ranges from five to seven years, and pubertal onset begins

between 2 and 3 months of age (Lee et al., 2004; Hummer et al., 2007).

Additionally, degus are a slow developing diurnal rodent whose circadian rhythm

developmental time course and sexual maturation have been well studied (Jechura

et al., 2000; Lee et al., 2004; Hummer et al., 2007). Previous studies

demonstrated that degus are diurnal based on locomotor activity data in the

laboratory and field (Goel &Lee, 1995 ; Kenagy et al., 1999), core temperature

rhythms (Goel &Lee, 1995), cortisol rhythms (Mohawk &Lee, 2005) and adaptations of the visual system (Jacobs et al., 2003).

Sex differences in degu physiological parameters are evident from previous data (Lee, 2004). During adolescence, hormones facilitate changes in circadian phase (Hagenauer dissertation, 2010), amplitude and period of this species and these changes are not complete until almost one year of age (Hummer et al., 2007). Male degus exhibit greater changes in chronotype as a result of gonadal hormones (Hummer et al., 2007; Hagenauer et al., 2009), which is consistent with data from studies utilizing humans and other rodents (Roenneberg et al., 2004; Hagenauer et al., 2009).

Research has also provided evidence for diurnality of the sleep/wake cycle by behavioral sleep analysis (Goel & Lee, 1996; Gaus et al., 2002). Conversely, the only published degu sleep study utilizing electrophysiological parameters found degus largely exhibited crepuscular sleep-wake behavior under baseline conditions (Kas & Edgar, 1998). This is in stark contrast to field studies, which demonstrated degus exhibit crepuscular rhythms during the summer due to low tolerance of high daytime temperatures (Garcia-Allegue et al., 1999) and attempt to minimize temperature stress in their natural environment (Fulk, 1976). During cooler periods of the year they are active throughout the day (Kenagy et al., 1999), and no reports exist of them exiting their burrows or being caught during the dark. Interestingly, access to running wheels contributes to their crepuscular activity in the laboratory. Kas and Edgar (1999) found that wheel access could invert the chronotype of male degus from

diurnal to nocturnal in many animals. These data demonstrate the complexity of the degu circadian system and highlight the variety of environmental parameters that may influence the timing of sleep. However, the sex differences in phase preference in degus is analogous to humans and provides additional rationale for examination of sex differences in degu sleep. Flexible chronotype (namely diurnal) and strong bimodal activity are strong commonalities among degus and humans (Kas & Edgar, 1999; Aschoff, 1967; Aschoff, 1968). *Thus, degus are a good additional diurnal animal model for basic sleep research.*

Conclusion

Collectively, because of these data, degus appear to be a diurnal species in which the study of sleep could be informative. My experiments aimed to explore the homeostatic and circadian sleep components in this animal model between sexes and across development during baseline and deprivation conditions. While several sleep studies have been performed in diurnal animals (i.e. squirrel monkeys, grass rats, chipmunks and ground squirrels), the research focused exclusively on male animals and did not address sleep during early development or following phase shifts of the light cycle (Dijk et al., 1989b; Edgar et al., 1993; Schwartz & Smale, 2005). My work aims to address these topics for the first time in a diurnal rodent. In Chapter II, I examine the baseline sleep characteristics of intact male and female young adult degus. Many sleep parameters are sexually dimorphic and I hypothesized that sleep would differ between sexes, with females demonstrating an increased amount of NREMS and delta power compared to

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males. In Chapter III, I examine the impact of sleep loss (via a sleep deprivation protocol) with both sexes. Additionally, I explore the interaction of sleep deprivation and circadian phase on recovery sleep within male and female degus. In Chapter IV, I examine sleep across development (specifically adolescence). Based on previous data, I expected male degus to demonstrate greater adolescent sleep changes than females across adolescence. Therefore, male juvenile degus were used exclusively in the developmental experiments. Sleep studies were performed during early adolescence and late adolescence under baseline and sleep deprived conditions. Finally, in Chapter V, I summarize my findings and further examine the results of the sleep studies, discussing the sex and age differences found within the animal model. Additionally, I propose a possible neuroanatomical framework that may facilitate these differences. Finally, I discuss the data in relation to current models of sleep regulation and provide suggestions for future directions of research.

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Figure 1.1

An EEG recording (10 second window adapted from Kas & Edgar, 1998)

demonstrating sleep and wakefulness of the diurnal rodent, *Octodon degus*. A)

NREMS is characterized slow, large amplitude EEG waves and low EMG activity

during recorded sleep. B) REMS consists of mixed frequency (theta dominated),

low amplitude EEG waves and muscle atonia (no EMG activity). C)

WAKEFULNESS is represented during the recording window, with a mixed

amplitude and high frequency EEG shown along with high muscle tone denoted

by EMG trace.

Figure 1.1
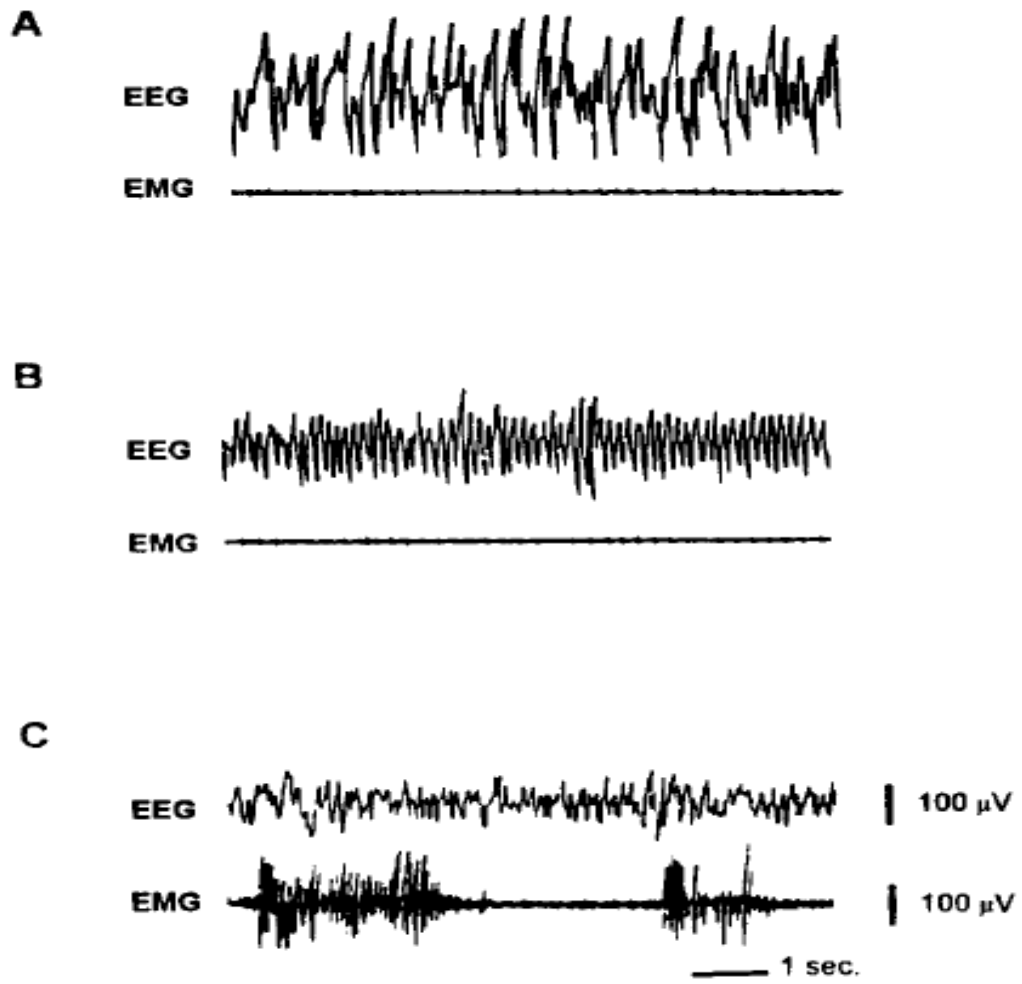
Figure 1.2

The two process model of sleep-wake regulation developed by Borbely (1982; adapted schematic). Borbely hypothesized the sleep-wake cycle was regulated by the interaction of two components (the homeostatic process "Process S" and the circadian process "Process C"). Process S, commonly referred to as homeostatic sleep drive, mediates the increase in sleep "need" due to extended wakefulness within animals, peaks during the early night, and dissipates across the predominant sleep phase, while the circadian component (Process C) consists of a biologically driven system that promotes arousal alternatively. The circadian and homeostatic components interact to mediate regulation of the sleep-wake cycle.
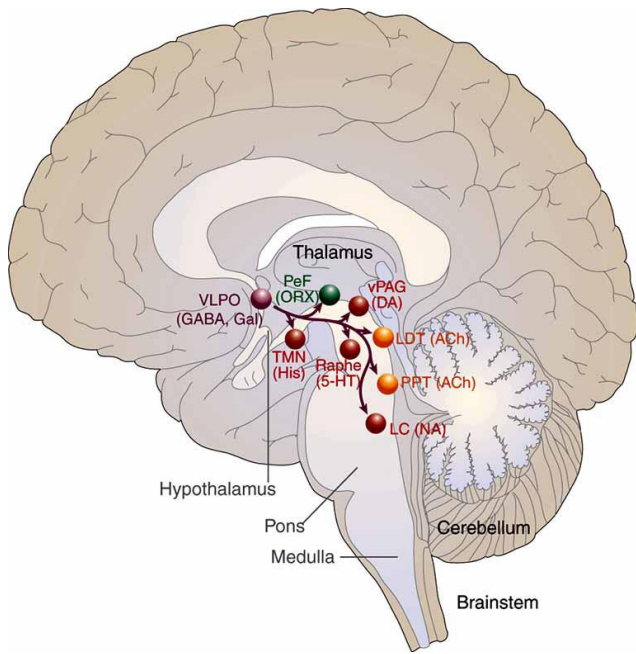
Figure 1.2
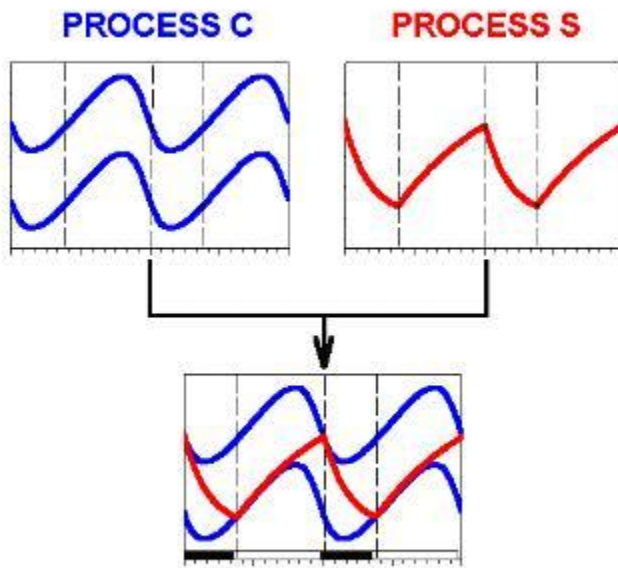
Figure 1.3

The generation and regulation of the sleep-wake cycle schematic diagram in humans (adapted from Saper et al., 2005). A) The descending network (purple line) of brain areas (colored dots) denote the neuronal systems involved in mediating NREMS and REMS. B) The ascending network (red line) of brain areas (colored dots) demonstrates the neuroanatomy systems that collectively regulate wakefulness in animals. These two large brain systems interplay to generate sleep and wakefulness alternately. Brain area abbreviations: Cholinergic (Ach) cell groups, basal forebrain (BF), dopamine (DA), gamma-aminobutyric acid (GABA), galanin (Gal), histamine (His), serotonin (5-HT), locus coeruleus (LC), laterodorsal tegmental nuclei (LDT), lateral hypothalamus (LH), melanin-concentrating hormone (MCH), noradrenaline (NA), orexin (ORX), perifornical (PeF), the pedunculopontine (PPT), tuberomammillary nucleus (TMN), ventrolateral preoptic nucleus (VLPO), and ventral periaqueductal (vPAG).

Figure 1.3

A

B

34
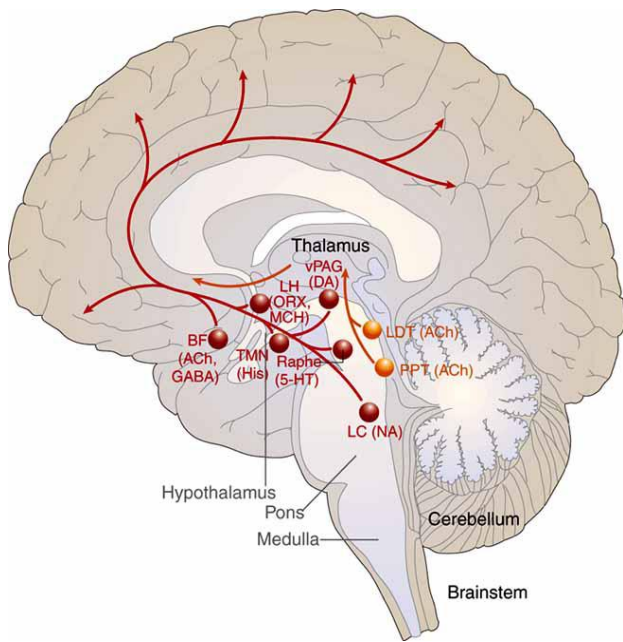
Figure 1.4

The human sleep/wake cycle across the lifespan (adapted from Roenneberg et al., 2004). Sleep dominates during infancy and early childhood of humans, with a majority of time spent in this behavioral state. REMS emerges as the most prevalent sleep stage during infancy and early childhood, while NREMS becomes the predominant sleep stage as aging progresses. The most dramatic changes in sleep duration and architecture occurs during adolescence and continues into young adulthood. Both sleep stages dissipate as aging occurs, with REMS declining more rapidly than NREMS. Sleep amount continues to decline across the lifespan and plateaus during old age.

Figure 1.4
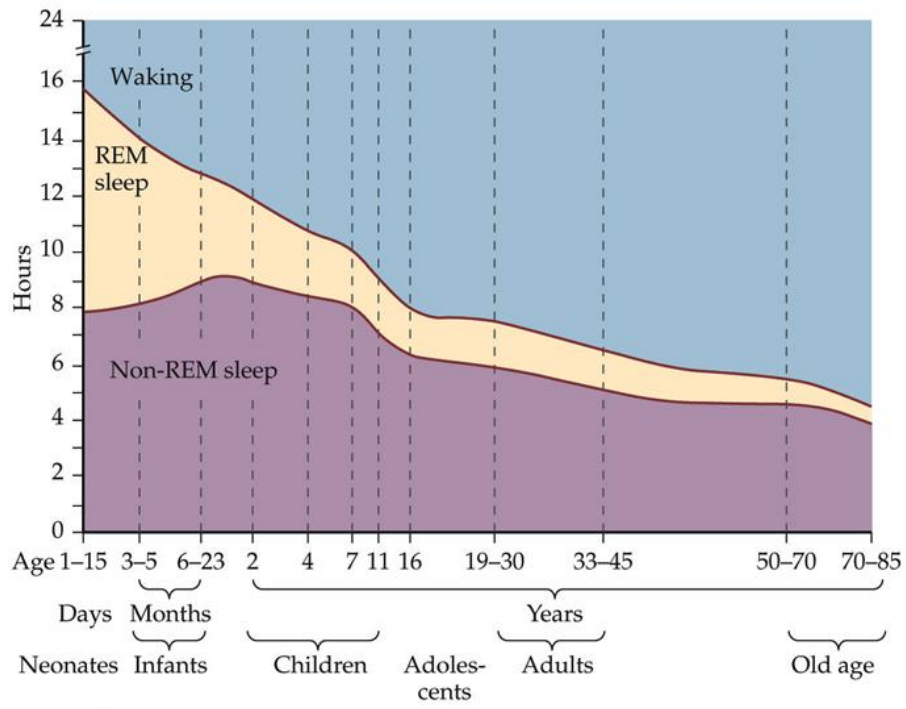
Chapter II
Sex differences in sleep patterns of diurnal *Octodon degus*

Abstract

Previous field and behavioral sleep studies have demonstrated that degus have a preference for diurnal activity and nocturnal sleep. However, very little work has examined sleep electrophysiologically in diurnal rodents, especially within both sexes. This study used electroencephalograph (EEG), brain temperature, and locomotor activity to investigate the homeostatic and circadian components of sleep in the diurnal rodent, *Octodon degus*. Animals were implanted with screw electrodes and a brain thermistor to collect electrophysiological sleep data. Male degus displayed more non-rapid eye movement sleep (NREMS) and consolidation during their dark period (night), which was consistent with prior studies utilizing locomotor activity and behavioral sleep. Female degus did not display differences in NREMS across the 24h recording period, yet they did have more NREMS consolidation during the night (as evidenced by longer NREMS bout duration). Additionally, females displayed increased delta power (indicated by slow wave activity, SWA) during the night,

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while males exhibited higher levels during the day. Neither sex demonstrated

circadian variation in rapid eye movement sleep (REMS), yet males had

significantly higher REMS across both the day and night compared to females.

Both sexes demonstrated increased activity around the light/dark transitions

(crepuscular activity), indicating the strong circadian arousal during dusk and

dawn time periods. These data demonstrate that sleep is sexually dimorphic in

the degu and may provide some insight into how sex impacts sleep in a diurnal

mammal.

Introduction

Sleep is defined as a reversible behavioral state that is ubiquitous across

species and necessary for many vital life processes. The exact function of sleep,

however, has been the subject of longstanding debate and is still being

investigated. Nevertheless, sleep is demonstrated to play a large role in immune

function, learning and memory consolidation, emotional regulation, hormonal and

endocrine secretion, as well as cognitive and behavioral functioning (Turner et

al., 2007; Opp & Toth, 2003; Steiger, 2003; Walker et al., 2002; Sharma &

Mazmanian, 2003; Tsuno et al., 2005; Gais et al., 2000). Sleep also differs

between sexes, which has also generated interest in the sexually differentiated

patterns of sleep and what role this ultimately plays in sleep function.

Rechtschaffen and Kales (1968) characterized sleep into distinct stages

using electrophysiological criteria. Sleep consists of two main stages: non rapid

eye movement sleep (NREMS) and rapid eye movement sleep (REMS).

NREMS is typified by low frequency, high amplitude EEG and little or no muscle tone. NREMS is further distinguished into substages of light sleep and deep sleep (Slow Wave Sleep). Light sleep stages are associated with sleep spindles, and deep sleep stages (SWS) are associated with slow oscillating waveforms, namely delta waves (0.5-4.5Hz). Delta power (slow wave activity,SWA) is widely used as an indicator of homeostatic sleep pressure. In contrast, REMS is characterized by high frequency, low amplitude EEG with no muscle tone and rapid eye movements.

Sex differences within many species in these sleep stages are described in previous research (Fang & Fishbein, 1996; Manber & Armitage, 1999; Shechter & Boivin, 2010). Research in humans has established that females display more NREMS, higher levels of delta power within slow wave sleep (SWS), and greater sleep spindles in early sleep stages (Dijk et al., 1989; Manber & Armitage, 1999; Armitage & Hoffmann, 2001). Additionally, women exhibit higher levels of sleep power density over a wide frequency range during REMS (Dijk et al., 1989). Further, studies have highlighted that women show a slower age-related decline in delta power compared to their male counterparts, and therefore, sexually dimorphic sleep characteristics become more apparent with advancing age (Manber & Armitage, 1999; Koehl et al., 2006).

Results of nocturnal rodent sleep studies have generated mixed findings on sex differences. Rat males and females exhibit similar amounts of total sleep and NREMS, yet males have REMS significantly more than females across the light dark cycle (Yamaoka, 1980; Fang & Fishbein, 1996). Conversely, studies

examining baseline sleep patterns of mice showed females spend less time in NREMS and REMS than males (Koehl et al., 2006). These contradictory findings illuminate the need for more studies examining these sex differences across diurnal and nocturnal species and the necessity for understanding what role hormones play in sleep regulation.

Researchers have posited sex hormones exert a strong influence on sleep physiology and may account for these sexually dimorphic characteristics. Estrogen increases norepinephrine within sleep promoting brain areas like the brainstem, hypothalamus, locus coeruleus, which enables noradrenergic neurons to suppress REMS in rats (Fang & Fishbein, 1996). Females have more neurons within the locus coeruleus, an area directly involved in REMS suppression in nocturnal species (Manber & Armitage, 1999). Furthermore, studies utilizing gonadectomized and hormone treated animals have further elucidated the role hormones play in sleep-wake regulation.

In rats, progesterone suppressed REMS and decreased wakefulness and shortened latency to NREMS (Gandolfo et al., 1994; Lancel et al., 1996). Ovariectomized female mice displayed decreased wakefulness and increased NREMS compared with intact controls (Paul et al., 2006). Testosterone also plays a role in REM sleep regulation in mice (Fang & Fishbein, 1996; Manber & Armitage, 1999). Neonatal castration increased REMS in adult mice (Fang & Fishbein, 1996), while adult castration did not elicit any changes in sleep-wake patterns (Paul et al., 2006). Additionally, neonatal administration of exogenous testosterone reversed this phenomenon in castrated adult male mice and

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decreased REMS in female neonatal mice (Branchey et al., 1973). This work suggests hormones may have a critical window during perinatal development to alter sleep patterns in male and female rodents. Early steroid exposure may organize sleep patterns in other species as well, but this has not yet been shown.

The aforementioned work underscores the need for more studies on sex differences in sleep, as this topic has been largely ignored and has only recently been investigated again. Although a hormonal role in adult sleep regulation has been revealed in previous studies, the mechanisms behind species-specific hormone effects remains unclear. Estrogen suppresses REMS in nocturnal rodents, yet in humans, administration of exogenous estrogen increases REMS, sleep latency and sleep efficiency (Thomson & Oswald, 1977; Schiff et al., 1979; Fang & Fishbein, 1996). Clearly, research suggests differential hormonal effects on sleep among diurnal and nocturnal species, yet the number of species studied is too small to reach any firm conclusions. Baseline sleep has been examined in several diurnal species including the grass rat, chipmunks, and squirrel monkeys (Dijk & Daan, 1989; Edgar et al., 1993; Schwartz & Smale, 2005), but these sleep studies have not included females. Because sleep exhibits different sexually dimorphic characteristics across nocturnal and diurnal chronotypes, investigating the sleep of both sexes in a diurnal species could be especially relevant to understanding the sex differences in human sleep.

Octodon degus (degu) are an excellent animal model for these studies. Degus are a long-lived, diurnal, and social species (Goel & Lee, 1996; Lee, 2004) with a relatively long developmental period. Previous studies demonstrate

that degus are diurnal based on locomotor activity data in the laboratory and field (Goel & Lee, 1995; Kenagy et al., 1999), core temperature rhythms (Goel & Lee, 1995), cortisol rhythms (Mohawk &Lee, 2005) and adaptations of the visual system (Jacobs et al., 2003). Additionally, Mendelson (1982) published an abstract that reported clear evidence of diurnality of sleep/wake patterns using EEG, but this work was left unpublished in its entirety. Behavioral sleep studies similarly demonstrated that degus sleep more during the night (Goel & Lee, 1995; Gaus et al., 2002). However, the only previously published sleep paper utilizing EEG in degus suggested they were crepuscular (Kas & Edgar, 1998). Several factors may account for this: wheel-running has been shown to invert these animals chronotype from diurnal to nocturnal (Kas & Edgar, 2000) and ambient temperature alters their activity patterns (Fulk, 1976; Kenagy et al., 1999; Lee et al., unpublished). The study described here housed animals without running wheels and maintained housing chambers at an ambient temperature that allows natural diurnal activity. Additionally, our study utilized female degus to examine sleep for the first time and compared them to their male counterparts for further analysis.

Female degu circadian rhythms are well characterized and they share many characteristics with humans (Lee, 2004). In addition, female degus have a relatively long estrus cycle for rodents (~21 days) which is more analogous to humans (Labyak, 1993; Labyak & Lee,1995) than that of laboratory nocturnal rodents (~4-7 days), allowing for examination of intact female degu sleep less contaminated by the frequent high activity typical of estrous (Marrone et al.,

1976; Kent et al., 1991). As previously discussed, hormones have been implicated to play a role in sleep regulation and may be vital to understanding the sex differences in sleep. Previous diurnal animal studies have exclusively studied sleep in male populations (Dijk & Daan, 1989; Edgar et al., 1993; Schwartz & Smale, 2005). Thus, using both male and female degus will ultimately allow investigation of questions about mechanisms of hormonal influences on female sleep patterns and the underlying neurobiological circuitry.

Therefore, this study focused on various sleep components under baseline conditions in male and female populations of the diurnal rodent, the *Octodon degus*. I collected twenty-four hours of baseline sleep recordings and examined its architecture, depth and timing. If degu sleep patterns were more homologous to diurnal humans than nocturnal rodents, they would provide an additional animal model enabling further examination of the underlying neurobiology of sleep in diurnal mammals. Moreover, these experiments allowed us to begin to investigate the role hormones may play in sleep regulation in this diurnal species. I hypothesized that male and female degus would sleep more during the dark period than light, and females would exhibit increased NREMS (both in amount and depth) relative to their male counterparts. Additionally, I expected males to have more REMS and less delta power than female degus based on the results of previous sex differences studies.

Methods

Animals and Surgery

Six adult male (1-3 yrs.) and eight adult female (1-2.5 yrs. of a 5.5yr lifespan) *Octodon degus* were used in this study. Degus were obtained from The University of Michigan colony. Animals were housed individually in recording chambers and maintained on a 12:12 light/dark cycle. Recording chambers [Nalgene cages (42.5 x 22 x 19cm)] were designed with a cover to accommodate a tether system that enabled collections of EEG, locomotor activity rhythms (via an infrared detection device), and brain temperature (via brain thermistor, Omega Instruments). Food (Purina 5001 Rodent Chow) and water were provided *ad libitum*. The ambient temperature was 18 ± 1°C.

Animals were anesthetized with 5% isofluorane, positioned in a stereotaxic device and surgically implanted with screw electrodes that allowed the collection of EEG recordings. Two frontal (+11.0 anterior from earbar zero, ± 3.0ML) and two occipital (+ 2.5 anterior from earbar zero, ± 3.0ML) epidural EEG leads were placed in position using stainless steel screws (Kas & Edgar, 1999a). A thermistor (enabling the monitoring of brain temperature) was implanted under the skull on top of the dura between the two sets of screws. All leads were connected to a pedestal autoclaved prior to surgery. The pedestal was fixed to the skull with dental acrylic after skull surface was dried and coated with Superglue. Body temperature of animals was maintained with a water-filled heating pad during surgery and post-operatively until arousal. The surgical opening was subsequently covered with Nolvasan antiseptic ointment, and animals were given 3-4cc of Lactated Ringer's Solution subcutaneously (s.c.) following surgery to prevent dehydration. Prophylactic antibiotic was given postoperatively (20mg/kg

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chloramphenicol, s.c.) for 4 days. Possible postoperative pain was managed with butorphinol (0.10 mg/kg intraperitoneal [i.p.] preoperatively and 0.05 mg/kg postoperatively s.c). The animals were also given a post-operative one-time injection (s.c) of dexamethasone (0.2 mg/kg) to reduce swelling post-operatively. The condition of post-surgical animals was monitored twice daily for several days and pain medication (butorphinol) was administered as needed to alleviate discomfort. The animals were fed 1 ml of Nutri-cal (a high calorie supplement) and water twice daily during the post-operative period. Degus were weighed daily and animals were categorized as fully recovered when weight stabilized. Prior to beginning data collection, the degus spent at least two weeks habituating to the recording chamber, including being tethered for one week before collecting baseline EEG data. All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee.

Sleep data analysis

EEG was processed through an eight-channel Grass polygraph (Grass Technologies, West Warwick, RI). EEG was passed through filters (Coulbourn Instruments, LeHigh Valley, PA) and into a data acquisition system that samples, digitizes and stores the data. ICELUS, a computer-assisted visual scoring software (Mark Opp, Ann Arbor, MI), was used to categorize sleep states. Several measures were introduced into the program, including EEG, core brain temperature, and locomotor activity (LMA collected using an infrared device placed atop the recording chamber) to accurately score sleep states. All data were divided into 12-s epochs, which were characterized using the ICELUS program.

Three consecutive epochs (36s) were needed to classify a sleep state. NREMS was categorized by high amplitude, low frequency EEG signal with little or no locomotor activity. REMS was characterized by high frequency, low amplitude, theta dominated EEG signal with no locomotor activity. Wakefulness was characterized by mixed voltage, high frequency EEG signal with varying amounts of locomotor activity. Spectral analysis of sleep data was carried out. Delta power (sleep intensity as measured by slow wave activity [SWA]) during NREMS was quantified in the 0.5Hz-4.5Hz frequency band.

Statistical Analyses

Total NREMS, total REMS, spectral parameters (i.e. delta power) and sleep consolidation (determined by NREMS bout length) were compared between light and dark conditions. Analysis of variance (ANOVA) with post hoc *Tukey* analysis and *t tests* were used to determine significant differences in the sleep of animals between the light and dark period across sex.

Results

Table 2.1 illustrates the sleep architecture of male and female degus across the baseline recording period (24h). In addition to the significant sex differences in the amount of NREMS, REMS, WAKE, females exhibited significantly increased NREMS consolidation (as evidenced by longer NREMS bout duration) (t (334)= -6.751, p<.001) and decreased NREMS bouts (number/hour) (t (334)=4.491, p<.001) as compared to male degus (see Table 2.1). Additionally, females demonstrated a significant decrease in transitions during the night as compared to day (t (95)=2.173, p=.032). NREMS across baseline conditions (24h) showed

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sexually dimorphic patterns, with females demonstrating significantly more total

NREMS than their male counterparts with independent samples t-test analysis (t

(334)= -4.210, p<.001; Figure 2.1A). There were no day and night differences in

REMS amount across baseline recording for males (t (71)= -1.891, p=.063) or

females (t (95)= -.112, p=.911; Figure 2.1B), although males had significantly more

total REMS than females (t (334)=5.376, p<.001). In addition, males had

significantly more REMS than females during both the light (t (71)=2.419, p=.018)

and dark phase (t (71)=4.527, p<.001). Male degus displayed significantly more

total WAKE than females (t (334)= 3.321, p=.001; Figure 2.1C). No significant sex

difference in normalized delta power across the 24h baseline period were

observed for males and females (t (46)=.081, p=.777). However, males

demonstrated higher relative delta power during the day as compared to the night

(104.31± 1.03 vs. 95.69± 2.10, t (11)=3.130, p=.010; Figure 2.1D). Conversely,

females exhibited higher relative delta power during the night compared to the day

(105.39± 1.71 vs. 94.69± 1.36, t (11)= -6.379, p<.001; Figure 2.1D). Thus, males

demonstrated higher relative delta power during the day (t (11)=5.132, p=.000) and

females were higher during the night (t (11)= -3.669, p=.004).

Male degus exhibited significantly more NREMS during the night than the

day, when data were analyzed as day (12 light hours) versus night (12 dark hours)

using paired t-test (t (71)= -3.097, p=.003; Table 1). Females displayed no

significant differences in NREMS across the light-dark cycle (t (95)=.284, p=.778).

No difference in REMS distribution was observed across the day and night for

males or females, although more REMS occurred at night for both sexes (Table 1).

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In addition, WAKE was significantly increased during the day for males compared to the night (t (71)=3.269, p=.002), which is expected due to its inverse relationship with sleep (Table 1). Females demonstrated no significant differences in WAKE amounts across the day and night (t (95)= -.550, p=.583). NREMS bout duration did not differ across the day and night for males (t (71)=1.53, p=.052) or females (t (95)= -.977, p=.331; Table 1).

In addition to examining sleep across the light/dark cycle, sleep was divided into multi-hour periods for further analysis based on circadian activity, enabling further analysis of sleep-wake patterns (Figure 2.2A). Block 1 included 2h light/dark transition periods (ZT1/24, 12/13), during which Kas and Edgar (1998) found that degus rarely slept. Block 2 (ZT2 & ZT11) was 1h periods after or before the highly active transitions, when degus increased sleep time above the 24h mean. Block 3 was the remainder of the day (ZT3-ZT10), which consisted of the remaining light phase. Block 4 was the remaining dark phase (ZT14-ZT23). There were significant differences in NREMS distribution observed across time blocks using one-way between subjects ANOVA (F(3,116)=2.783, p=.044; Figure 2.2B). Further post hoc comparisons demonstrated there was significantly less NREMS during the transitions than the night for males and females (p=.032).   In addition, when male NREMS was analyzed across time blocks using within subjects ANOVA, significant differences were found (F(3, 140)=6.774, p<.001). Post hoc comparisons revealed males sleep significantly less during the transitions (p=.032) and the day (p<.001) compared to the night. Additionally, the data demonstrated significant differences in NREMS bout duration across time blocks for males

(F(3,140)=2.979, p=.038; Figure 2.2C). Sleep was more consolidated (as evidenced by longer NREMS bout lengths in our study) during daytime naps and the night compared to the day for male degus (p=.05 and p=.048, respectively). Sleep consolidation was relatively high for females across transitions, naps, day, and night, showing no significant changes as a function of time period (F(3,188)=2.27, p=.555). Thus, sleep consolidation was higher during the night and daytime naps for males, yet for females, sleep was fairly consolidated across all time blocks and was not influenced by circadian phase.

Discussion

The data demonstrate that male degus produce a typical diurnal sleep pattern, producing more NREMS during the dark phase. NREMS revealed strong circadian variation in male and female degus alike, with light dark transitions mediated most strongly by circadian influence. NREMS consolidation was sexually dimorphic, with females exhibiting significantly higher overall sleep consolidation and fewer transitions than their male counterparts. Furthermore, females showed fewer transitions during the night as compared to their day, which may demonstrate higher sleep efficiency in females during the night sleep phase. REMS did not reveal any circadian variations in either sex, although both groups did spend more time in REMS during the night. In addition, delta power demonstrated sex differences, with higher relative levels observed in males during the day and in females during the night. Females also demonstrated an apparent slower decline in delta power across the night than their male

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counterparts. Thus, I conclude that both male and female degus have sleep adaptations to a diurnal niche, but they are distinctly different adaptations.

In this descriptive study, my aim was to characterize degu sleep across the light dark cycle during baseline conditions for male and female degus. Previous field studies have demonstrated degus have a diurnal activity preference (Fulk, 1976). However, very little work has examined sleep in diurnal rodents and there was no consensus on degu sleep from previous studies. Despite using male degus, Kas & Edgar (1998) found no significant circadian variation of sleep across the light dark cycle, which may be due to different housing conditions and varying ambient temperature during the experiments (at 21⁰C compared to 18⁰C in our experiments). Previous studies have illustrated that degu activity and sleep are sensitive to environmental temperature, with higher temperatures driving degus toward more crepuscular and nocturnal patterns (Garcia-Allegue et al., 1999; Lee et al., unpublished data). Additionally, Kas & Edgar (1999b) later discovered that access to running wheels could alter degu chronotype from diurnal to nocturnal. In this study, our animals were housed without wheels and in a cooler ambient temperature (18°C) than reported by Kas & Edgar (1998) to reduce environmental factors that drive the degu towards crepuscular behavior. Under these conditions, male degu activity and sleep were consistent with behavioral reports of diurnality. Males slept significantly more during the night and displayed increased activity around the light/dark transitions and during portions of the light phase. Interestingly, this latter feature is also clear in the data of Kas & Edgar (1998), suggesting that high

activity during light/dark transitions ("dawn" and "dusk" respectively) is the most stable feature of degu sleep/activity rhythms in both sexes.

Previous work examining sleep within this species has utilized male degus exclusively (Kas & Edgar, 1998; Kas & Edgar, 1999a; Kas & Edgar, 2000). We were interested in whether degu sleep displayed sexually dimorphic characteristics during baseline conditions. This study was the first to examine sleep in female degus utilizing EEG. Females have significantly increased amounts of NREMS and decreased REMS compared with their male counterparts, as hypothesized. Overall, REMS amount in degus were quite low, and females exhibited significantly lower levels than males. Due to the reported role of estrogen in the regulation of REMS (Fang & Fishbein, 1996), this was not entirely surprising. Estrogen has demonstrated a suppressive effect on REMS in nocturnal rodents (Fang & Fishbein, 1996), while humans have exhibited enhanced REMS levels in response to exogenous estradiol administration (Thomson & Oswald, 1977; Antonijevic et al., 2000). Interestingly, intact female degus appear to more closely mimic nocturnal rodents with regards to hormonal regulation of REMS. Thus, primates and rodents may differ in how steroids impact REMS.

A surprising finding was that female degu sleep patterns were not diurnal under baseline conditions, but largely reflected crepuscular activity. While female degus were expected to exhibit more overall NREMS as compared to males, the weak influence of circadian phase on sleep was unexpected. NREMS amount did not differ during the non-transition blocks, suggesting females lack

nocturnal sleep preference compared to their male counterparts. However, REMS, quantity of NREMS bouts and increased NREMS bout duration, and amount of transitions, while not all significantly different between night and day, are consistent with males and a preference for nocturnal sleep. Furthermore, females demonstrate less delta power during the day compared to males, indicating their sleep may be less efficient during the day, which may account for the higher percentage of the day spent in NREMS. Additionally, females produced higher levels of delta power during the dark phase compared to their own daytime levels and their male counterparts, which further illustrates a nocturnal preference for sleep within female degus.

Relatively recently, researchers have made significant strides in determining the impact of sex on NREMS. While it is widely accepted that sexually dimorphic patterns of sleep are mediated by hormonal regulation, recent studies are elucidating the mechanism by which this may occur (Crunelli et al., 2006). The thalamus generates the slowly oscillating delta waves and sleep spindles that are the hallmark of NREMS, and recent work has illustrated that T-type calcium (Ca^{2+}) channels facilitate generation of these waveforms (Lee et al., 2004). Mice lacking a functional subunit of the thalamic T-type Ca^{2+} channels have decreased NREMS and sleep spindles (Crunelli et al., 2006). Furthermore, estrogen upregulates T-type Ca^{2+} channels within the hypothalamus and pituitary (Qiu et al., 2006). This lends support to the idea that estrogen upregulated Ca^{2+} channels may mediate the increased NREMS levels among female degus and may possibly play a role in sleep consolidation.

Sleep consolidation was relatively high for degus compared to the more widely studied nocturnal rodents (Guzman-Marin et al., 2006). Degus NREMS bout length were over twice as long as their nocturnal counterparts for males and four times as long for females (Guzman-Marin et al., 2006). In addition, NREMS consolidation was sexually dimorphic, with female degus displaying higher NREMS duration than males during all time periods. Moreover, females exhibited significantly less NREMS bouts and transitions, which point to increased sleep efficiency over males. Hormones have been implicated in the regulation of sleep consolidation and efficiency in humans and our findings support this theory. However, hormones have not been implicated in all sexually dimorphic patterns of sleep, specifically delta power levels.

Some research suggests delta power (as measured by SWA within NREMS) is regulated independently of hormones (Driver et al., 1996; Lamarche et al., 2007), yet most human and rodent studies consistently demonstrate females have higher levels of delta power than males (Wever, 1984; Mourtazaev et al., 1995). This begs the question of what may be underlying these homeostatic differences between sexes, if not hormonal influences? Tononi & Cirelli (2006) posited the function of NREMS is directly linked to synaptic homeostasis. This synaptic homeostasis hypothesis asserts that increased sleep pressure (indicated by higher SWA levels in NREMS) was caused by increased synaptic strength. Recent experiments within this research group have supported this hypothesis, finding synaptic plasticity directly mediates subsequent sleep homeostasis (Esser et al., 2007; Vyazovskiy et al., 2007;

Vyazovskiy et al., 2008; Tononi, 2009). Synaptic homeostasis may be sexually

dimorphic and account for the known sex differences in sleep homeostasis in a

variety of species, yet much work remains to be done in this burgeoning research

area. Future work needs to manipulate hormone levels in both sexes, with one

group lacking the primary steroid hormone (testosterone or estrogen) and being

compared to its own control group (a group with normal estrogen/testosterone

levels).

There are several possible limitations of our study. Firstly, only collecting

24h of baseline data in a species with high inter-individual variability of activity

patterns might limit the significance of results. However, our sampling method

was sufficient to observe differences in NREMS across the day-night cycle. In

addition, the only previous electrophysiological study of degus utilized the same

24h baseline recording period. Another possible limitation of our study is low

power due to the small number of animals used in the study. However, the

sample size of the study is consistent with the previous EEG study in these

animals, further demonstrating the significance of our study results. In addition,

these animals also have a very low amount of REMS and relatively short sleep

bouts relative to primates. Yet, our results are consistent with the low amount of

REMS previously found in these animals (Kas &Edgar, 1999a).

Degus spend a very small percentage of time in REMS and showed great

variability between animals than NREMS, which may account for the lack of

significant differences across circadian phase for both sexes. Despite utilizing

females in sleep data collection during only non-estrous periods, this inter-

individual variability was greater in female degus than males. My study utilized intact female degus and possible heterogeneity of hormone levels across the luteal ovarian phase may have played a role in the large variability within females.

In conclusion, my study has illustrated the overall diurnal nature of degus sleep wake patterns and highlighted the sexually dimorphic characteristics of their sleep-wake patterns. This study is the first to characterize degu sleep as diurnal utilizing EEG and examine baseline sleep of female degus in relation to males. My animal model more closely resembles human NREMS (in amount and consolidation levels) as compared to more well studied nocturnal rodent species. Not only will this animal model allow us to investigate if sleep differences are mediated by diurnal/nocturnal chronotype, but the relatively long degu estrus cycle will enable investigation of intact, ovariectomized and hormone replaced females to more clearly elucidate the impact of hormones within adult sleep regulation. Sleep studies utilizing ovariectomized degus and estradiol/progesterone replacement are necessary to more fully understand the role hormones play in sleep regulation within this species. Additionally, future studies should examine how the circadian and homeostatic processes interact in degu sleep by utilizing sleep deprivation protocols.

Acknowledgements

Table 2.1: A table illustrating the sleep architecture of male and female degus under baseline conditions.

| Sleep Parameter by Sex	Light period	Dark period	Total 24h
Wake duration (% of recording time)			
Males	65.99 ± 2.2*	54.02 ± 2.9†	60.0 ± 2.2*
Females	50.27 ± 2.7	52.08 ± 2.5	51.2 ± 3.2
NREMS duration (% of recording time)			
Males	31.85 ± 2.1*	42.77 ± 2.6†	37.3 ± 1.9*
Females	48.59 ± 2.5	47.09 ± 2.4	47.8 ± 3.2
NREMS bouts (number/h)			
Males	6.13 ± 0.5*	6.00 ± 0.4*	6.1 ± 0.7*
Females	4.81 ± 0.3	4.29 ± 0.2	4.6 ± 0.1
NREMS bout duration (min)			
Males	3.39 ± 0.3*	4.05 ± 0.3*	3.7 ± 0.5*
Females	5.99 ± 0.4	6.49 ± 0.4	6.2 ± 0.1
REMS duration (% of recording time)			
Males	2.16 ± 0.4*	3.21 ± 0.6*	2.7 ± 0.9*
Females	0.83 ± 0.2	1.17 ± 0.8	1.0 ± 0.3
Transitions (number/h)			
Males	8.0 ± 0.5*	7.6 ± 0.5	7.8 ± 0.5
Females	9.9 ± 0.6	8.5 ± 0.5†	9.2 ± 0.9

*=Significant difference between sexes (p< 0.05)
†=Significant difference within sex (p< 0.05)

Figure 2.1

A) Percent of time spent in NREMS plotted across 24 hr during baseline conditions. Values denote the hourly NREMS mean ± standard error of the mean (SEM). Total NREMS was sexually dimorphic, with females demonstrating significantly higher levels of NREMS than males ($p < 0.05$). B) Percent of time spent in REMS plotted across 24 hr during baseline conditions. Values denote the hourly REMS mean ± standard error of the mean (SEM). Total REMS was sexually dimorphic, with males demonstrating significantly higher levels of REMS than females ($p < 0.05$). C) Percent of time spent in WAKE plotted across 24 hr during baseline conditions. Values denote the hourly WAKE mean ± standard error of the mean (SEM). Total WAKE displayed sexually dimorphic patterns, with males demonstrating significantly higher levels of WAKE than females ($p < 0.05$). D) Percent of time spent in DELTAPOWER plotted across 24 hr during baseline conditions. Values denote the hourly DELTAPOWER mean ± standard error of the mean (SEM). DELTAPOWER displayed sexually dimorphic patterns during the day, with males demonstrating significantly higher levels than females ($p < 0.05$). DELTAPOWER displayed sexually dimorphic patterns during the night, with females demonstrating significantly higher levels than males ($p < 0.05$). In all line graphs, the solid line indicates total electrophysiological state percentage (NREMS, REMS, WAKE, DELTAPOWER) of male degus across 24h and the dotted line represents female degus across 24h. The x-axis denotes time (hour) and the y-axis denotes WAKE (as a percentage of total recording time).

Figure 2.1
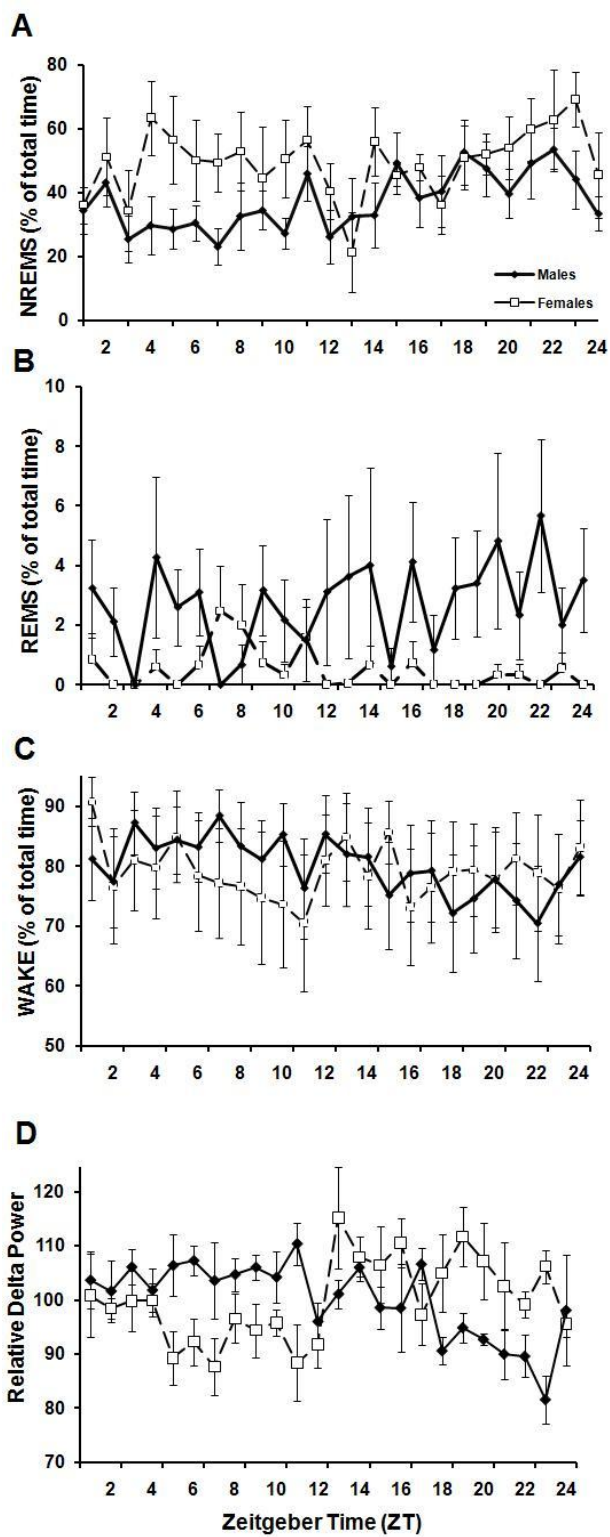
Figure 2.2

A) Percent of time spent in NREMS each hour plotted across 24 hr under baseline conditions. Values represent the hourly mean ± standard error of the mean (SEM). The solid line denotes total NREMS percentage of male degus and the dotted line represents females across 24h. There was a clear pattern of variation across the 24 hr that we divided into 4 periods: 2 h of transition around light changes (Transitions), daytime naps (Naps), 8h of the light phase (Day), and 10h of the dark phase (Night) to enable further analysis. B) NREMS across time blocks (transitions, naps, day, and night) during baseline recording for males and females. In all figures, bars represent the mean ± standard error of the mean (SEM). The black and white striped bars indicate transitions (average of ZT12-ZT13, ZT24-ZT1), the gray bars denote daytime naps (average of ZT2, ZT11), the white bars indicate daytime mean (average of ZT3-ZT10) and black bars denote the night mean (average of ZT14-ZT23). Asterisks denote statistically significant differences ($p < 0.05$). There was a significant increase of NREMS during the night as compared to transitions and day for males ($p < 0.05$) and transitions for females ($p < 0.05$). C) NREMS bout length/duration across time blocks (transitions, naps, day, and night) during baseline recording for males and females. In all figures, bars represent the mean NREMS bout length ± standard error of the mean (SEM). NREMS bout durations were longer during the naps and the night as compared to the day ($p < 0.05$) for males. Females demonstrated significantly decreased sleep consolidation during transitions ($p < 0.05$).

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Figure 2.2
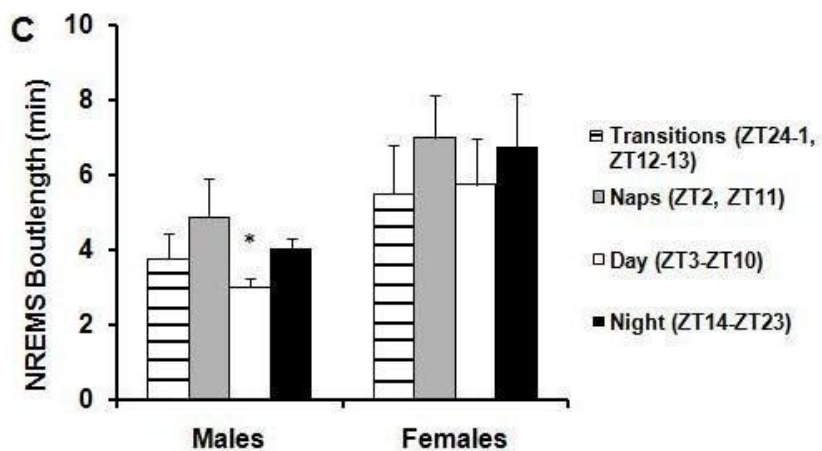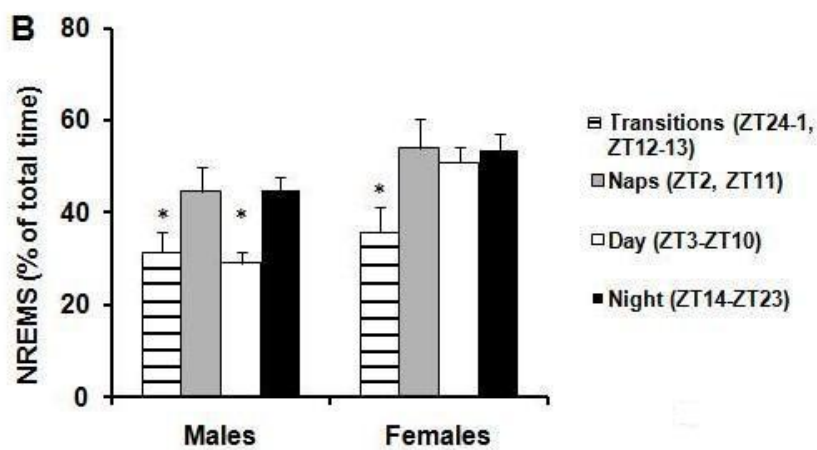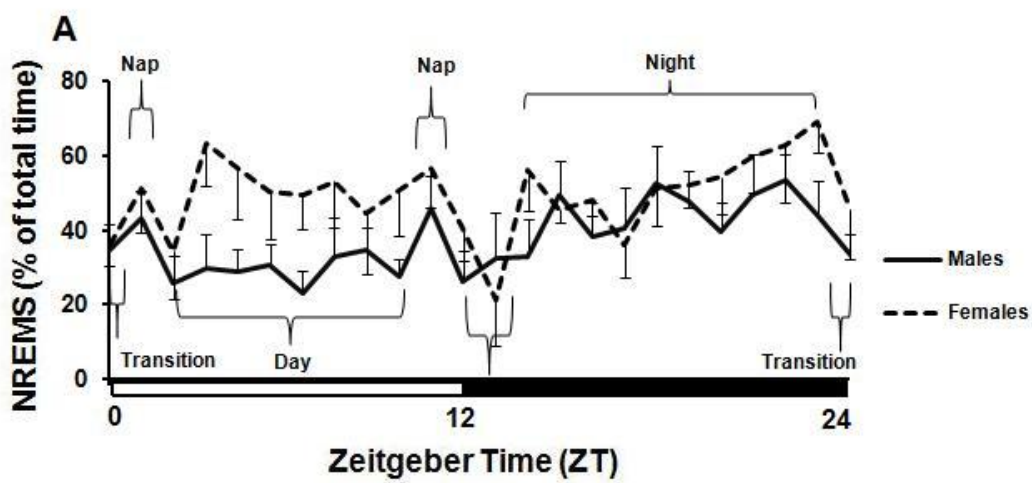
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Chapter III

Sex Differences in Recovery in Diurnal Degus Following Sleep Deprivation

Abstract

Previous sleep research has demonstrated sexually dimorphic sleep architecture across many species. And yet, very little work has examined sleep electrophysiologically in diurnal rodents, especially within both sexes following sleep deprivation. This study used electroencephalography (EEG), brain temperature, and locomotor activity to investigate recovery sleep following sleep deprivation in the diurnal rodent, *Octodon degus.* Following twenty-four hours of baseline recordings, animals were subjected to sleep deprivation for six hours during both the light and dark phases and forty-eight hours of sleep recordings were collected during recovery.   This enabled the investigation of compensatory mechanisms for recovery of lost sleep. Male degus displayed increased NREMS during recovery in both the day and night conditions, but the effects were more pronounced following night sleep deprivation. NREMS consolidation increased because of the night, but not day, sleep deprivation. Female degus

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demonstrated delayed increases in recovery NREMS amount and consolidation (during the end of the following day after night sleep deprivation). Degus have increased activity around the light/dark transitions, and this crepuscular activity was preserved across sleep deprivation conditions (although it was delayed after the night deprivation). This suggests that during light/dark transitions, the circadian drive for arousal is more powerful than the homeostatic sleep drive in degus and may provide some insight into how these processes interact in a diurnal mammal.

Introduction

Sleep is the ubiquitous process organisms within the Animal Kingdom need to survive. Sleep has a critical role in immune function, memory, hormone regulation and a host of other physiological and cognitive functions (Gais et al., 2000; Walker et al., 2002; Opp & Toth, 2003; Steiger, 2003; Turner et al., 2007). The prevalent model of sleep posits this physiological state as being regulated by two primary processes: a homeostatic drive and a circadian drive for sleep (Tobler & Borbely, 1986). Numerous studies of circadian and homeostatic components of sleep have sought to clarify how these systems interact to regulate the sleep/wake cycle in several animal models (Borbely et al., 1989; Tobler & Franken, 1993; Deboer et al., 1994). However, most research has been performed on nocturnal rodents. Very few studies have elucidated how these sleep mechanisms may interact in diurnal species due to their expense and difficulty in breeding.

Homeostatic and circadian mechanisms work in concert to regulate the timing, amount and intensity of the sleep-wake cycle. The suprachiasmatic nucleus (SCN) modulates the circadian drive for sleep (Edgar et al., 1993) by mediating the timing of sleep (Easton et al., 2004). Previous work examined the circadian drive to sleep by lesioning the SCN. Sleep time increases and becomes arrhythmic demonstrating the role of circadian rhythms in maintaining arousal (Edgar et al., 1993; Mendelson et al., 2003). While the anatomical site of the homeostatic drive has yet to be conclusively elucidated, it is likely mediated by the ventrolateral preoptic area (VLPO) and an accumulation of sleep regulatory substances (SRS) such as adenosine (Saper et al., 2005). NREM delta power (0.5-4.5 Hz) is a commonly used electrophysiological indicator of sleep intensity, which correlates with homeostatic pressure to sleep. NREMS and delta power increases significantly as a result of sleep deprivation (the paradigm commonly used to examine the homeostatic drive; Klerman et al., 1999).

Human studies have illustrated that sleep deprivation increases NREMS, REMS and delta power during recovery, while decreasing sleep latency and wakefulness (Borbely et al., 1981; Schussler et al., 2006). Rat deprivation studies have also highlighted the importance of sleep deprivation duration in determining subsequent recovery sleep response (Tobler et al., 1983; Tobler & Borbely, 1990). Deprivation duration increases homeostatic drive response, which affects the interaction of the circadian and homeostatic components of sleep during recovery (Tobler & Borbely, 1986; Trachsel et al., 1986).

Additionally, circadian phase also influences the impact of the sleep deprivation, with deprivation during the predominantly restful phase producing markedly increased sleep responses (Tobler & Borbely, 1986; Vyazovskiy et al., 2007).

Within diurnal animals, markedly different responses than their nocturnal counterparts are reported. For example, chipmunk demonstrate 300% more sleep within the dark period than the light, yet following a 24h sleep deprivation, wakefulness and NREMS remained unchanged, with REMS showing a slight enhancement (Dijk & Daan, 1989). The most prominent difference was a significant increase in delta power within NREM, indicating increased sleep intensity during recovery (Dijk & Daan, 1989). In contrast, squirrel monkeys, a diurnal primate, exhibited increased levels of NREMS and delta power following sleep deprivation during the dark period (Klerman et al., 1999). In addition, primate sleep deprivation research revealed a strong circadian influence on sleep timing and a much weaker homeostatic influence on sleep after deprivation than previously observed in humans (Klerman et al., 1999). These results highlight the complex nature of sleep mechanisms following deprivation, illuminating the high inter-species variability of these opponent processes interaction.

In addition to circadian and homeostatic factors playing an important role in sleep regulation and deprivation recovery, the impact of sex is a much less explored factor influencing rebound sleep architecture and depth, despite the differences in normal sleep. Human studies have produced mixed findings; studies of normal sleep demonstrated that females exhibit more total NREMS than males, yet the differences within sleep architecture were not significant

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under baseline conditions (Dijk et al., 1989; Achermann, 2004). Upon examining

spectral characteristics of sleep, females showed significantly more delta power

within NREMS and exhibited higher levels of sleep power density within REMS

(Dijk et al., 1989). Conversely, other studies demonstrate women have more

NREMS, higher levels of delta power and increased amount of sleep spindles

than their male counterparts within baseline conditions (Gaillard & Blois, 1981;

Wever, 1984b; Wever, 1984a). These results highlight the complexity of sex

differences within undisturbed sleep.

Increased homeostatic pressure enhances sex differences in sleep

regulation and architecture. Human females have a markedly greater response

to increased homeostatic drive, indicated by amplified intensity of NREMS and

longer duration of higher delta power levels following deprivation protocols than

males (Dijk & Beersma, 1989; Armitage & Hoffmann, 2001). Researchers have

posited that sex hormones are in part responsible, with sex steroid receptors in

sleep areas playing an important role in the sexually dimorphic recovery sleep

patterns (Manber & Armitage,1999; Shechter & Biovin, 2010). However, very

little work has been performed in other diurnal species examining these sex

differences under sleep deprivations conditions.

In nocturnal rodents, the results have been less clear, with different

species displaying different results of the impact of sex on sleep recovery. In

mice, there were no sex-specific differences in rebound sleep following

deprivation (Koehl et al., 2006). In rats, research has suggested sex specific

rebound sleep, with female rats demonstrating significantly higher levels of

NREMS (Andersen et al., 2008). Thus, the results appear to be species specific and may differ across nocturnal/diurnal boundaries.

To address this gap in the literature, the present study utilized a diurnal rodent, the *Octodon degus*. *Octodon degus* (degu) are a long-lived, diurnal, and social species (Goel & Lee, 1996; Lee, 2004) with a relatively long developmental period for a rodent. This animal model has been well characterized in circadian rhythms research. Field, laboratory and behavioral sleep studies have all underscored the diurnal nature of these animals (Fulk,1976; Mendelson, 1982; Goel & Lee, 1996; Kenagy et al., 1999; Gaus et al., 2002). In addition, sex differences in locomotor activity levels, circadian period, and development have been well studied in degus (Goel & Lee, 1995; Jechura et al., 2000; Lee, 2004; Hummer et al., 2007). The role sex hormones play in regulating circadian phenomenon has also been examined in this diurnal rodent (Jechura et al. 2000, 2003; Lee, 2004; Hummer et al., 2007). These factors serve to highlight degus as an ideal animal model to study sex differences in sleep, an area that has been largely ignored in diurnal rodent and nocturnal species alike.

This study aimed to investigate the sex differences of sleep following deprivation protocols in a diurnal rodent, with an emphasis on elucidating differences from nocturnal animal models. Utilizing the sleep deprivation paradigm, we examined the circadian and homeostatic interactions of degu sleep. The interaction of these factors were examined under increased homeostatic pressure conditions (via the 6h sleep deprivation paradigm) and between sexes.

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This study tested the hypothesis that circadian arousal components during the day, but not the night, were more powerful than increased homeostatic drive to sleep. Additionally, I hypothesized females would demonstrate higher NREMS and delta power levels during recovery compared to their male counterparts under the same conditions.

Methods

Animals and Surgery

Fifteen adult male (n=7) and female (n=8) *Octodon degus* (aged 1-3 yrs.) were used in this study. Degus were obtained from The University of Michigan colony. Animals were housed individually in recording chambers and maintained on a 12:12 light/dark cycle. Recording chambers [Nalgene cages (42.5 x 22 x 19cm)] were designed with a cover to accommodate a tether system that enabled collections of electroencephalography (EEG), locomotor activity (via infrared detection), and brain temperature (via brain thermistor). Food (Purina 5001 Rodent Chow) and water were provided *ad libitum*. The ambient temperature was 18 ± 1°C.

Animals were anesthetized with 5% isofluorane, positioned in a stereotaxic device and surgically implanted with screw electrodes that allowed the collection of EEG recordings. Two frontal (+11.0 anterior from earbar zero, ± 3.0ML) and two occipital (+ 2.5 anterior from earbar zero, ± 3.0ML) epidural EEG leads were placed in position using stainless steel screws (Kas & Edgar, 1999). A thermistor for monitoring of brain temperature was implanted under the skull on top of the dura between the two sets of screws. All leads were connected to a pedestal. The

pedestal was fixed to the skull with dental acrylic after skull surface was dried and coated with Superglue. Body temperature of animals was maintained with a water-filled heating pad during surgery and post-operatively until arousal. The surgical opening was subsequently covered with Nolvasan antiseptic ointment, and animals were given 3-4cc of Lactated Ringer's Solution subcutaneously (s.c.) following surgery to prevent dehydration. Prophylactic antibiotic was given postoperatively (20mg/kg chloramphenicol, s.c.) for 4 days. Possible postoperative pain was managed with butorphinol (0.10 mg/kg intraperitoneal [i.p.] preoperatively and 0.05 mg/kg postoperatively s.c). The animals were also given a post-operative one-time injection (s.c) of dexamethasone (0.2 mg/kg) to reduce swelling post-operatively. The condition of post-surgical animals was monitored twice daily for several days and pain medication (butorphinol) was administered as needed to alleviate discomfort. The animals were fed 1 ml of Nutri-cal (a high calorie supplement) and water twice daily during the post-operative period. Degus were weighed daily and animals were categorized as fully recovered when weight stabilized. Prior to beginning data collection, the degus spent at least one week habituating to the recording chamber, including being tethered before collecting baseline sleep recordings. All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee.

Sleep data analysis

EEG was processed through an eight-channel Grass polygraph (Grass Technologies, West Warwick, RI). EEG was passed through filters (Coulbourn

Instruments, LeHigh Valley, PA) and into a data acquisition system that samples,

digitizes and stores the data. ICELUS, a computer-assisted visual scoring

software (Mark Opp, Ann Arbor, MI), was used to categorize sleep states. Several

measures were introduced into the program, including EEG, core brain

temperature, and locomotor activity (LMA collected using an infrared device placed

atop the recording chamber) to accurately assign sleep states. All data were

divided into 12-s epochs, which were characterized using the ICELUS program.

NREMS was categorized by high amplitude, low frequency EEG signal with little or

no locomotor activity. REMS was characterized by high frequency, low amplitude,

theta dominated EEG signal with no locomotor activity. Wakefulness was

characterized by mixed voltage, high frequency EEG signal with varying amounts

of locomotor activity. Additionally, sleep consolidation (determined by NREMS

bout length) was also examined. Spectral analysis was also performed on the

sleep data recordings. Delta power (sleep intensity as measured by slow wave

activity [SWA]) during NREM sleep was quantified under the 0.5Hz-4.5Hz

frequency band.

Sleep Deprivation

Twenty-four hours of baseline sleep recordings were collected from male (n=7)

and female degus (n=7) before they were sleep deprived by gentle handling (6h).

In previous studies utilizing nocturnal rodents, sleep deprivation normally begins

immediately following lights off or immediately prior to lights on. To avoid their

naturally increased activity levels around dusk and dawn, degus were deprived

during the middle of the light (males only) or dark phases (ZT15-21 [mid-night] and ZT 3-9 [mid-day]). Baseline data recordings (24h) were collected prior to each treatment. EEG, temperature, and activity rhythms were collected for 48 hours during an uninterrupted recovery sleep period. Males, who received two deprivations, served as their own controls (pre- and post-experimental data were analyzed and compared) and the treatment order was counterbalanced. Males were given at least one week between deprivation protocols. All deprivation protocols resulted in significantly decreased NREMS and REMS amounts (less than 6% NREMS and no REMS; data not shown in figures).

Statistical Analyses

Total NREMS, total REMS, spectral parameters (i.e. delta power) and sleep consolidation (determined by NREMS bout length) were compared between baseline and treatment conditions. Analysis of variance (ANOVA) with post hoc *Tukey* analysis, and paired *t tests* were used to determine significant differences in the sleep of animals between the baseline and deprived conditions and between sexes for the night deprivation condition.

Results

6h Deprivation during Mid-day (ZT4-ZT9)

Using ANOVA, there were no significant differences in time spent in NREMS during the entire 15h of recovery following 6h of sleep deprivation during the light phase ($F(1,142)=1.266$, $p=.262$). Using post hoc analysis, NREMS in males significantly increased following the deprivation during the dark phase

(33.1± 8.7% and 45.8± 3.7%, p=0.03), 5h after deprivation ceased (Figure 3.1A).

Male degus displayed very low levels of REMS during baseline recording, and

during recovery, no change in REMS was evident (1.64± 0.33% and 1.53±

0.21%, respectively, data not shown). During the light/dark transition, the timing

and amount of activity was heightened and sleep amount was lessened during

both baseline recordings and post deprivation sleep recordings, highlighting that

the bimodal increased activity phenomenon is well preserved. Statistical analysis

revealed there were no significant differences in bout length of NREMS at any

time point following 6h of sleep deprivation during mid-day deprivation

(F(1,142)=1.266, p=.262; Figure 3.1B). Sleep deprivation during the light phase

did not affect relative delta power within recovery NREMS (F(1,73)=2.803,

p=.098; Figure 3.1C). Due to the minimal impact of day deprivation on recovery

sleep recordings, it was not repeated in female degus.

6h Deprivation during Mid-night (ZT16-ZT21)

Prior to the night sleep deprivation, total NREMS amount was comparable to

previously quantified baseline EEG characteristics in male and female degus

(37.70 ± 4.2% and 47.31± 5.3%, respectively). NREMS demonstrated no

significant differences over the 15h recovery period as compared to baseline for

both sexes (41.70 ± 6.6% and 39.21 ± 7.3%, respectively; F(1,356)=2.371,

p=.125). Additionally, there was no main effect of sex on NREMS levels

(F(1,356)=.219, p=.640), but a significant interaction of treatment and sex was

observed (F(1,356)=11.089, p=.001). In contrast, using repeated measures

ANOVA, there was a trend for an interaction between the time and condition (deprivation recovery versus control) within NREMS during the night in the first three hours post-deprivation (ZT22-24) for male degus ($F(1,38)=3.522$, p=.068; Figure 3.2A). The increased activity around the light/dark transition was delayed from ZT24 to ZT1 following the night deprivation in the males. Furthermore, there was an additional significant increase in NREMS during the following day between ZT2-ZT5 ($F(1,48)=5.00$, p=0.03). Analysis of male compensatory sleep response revealed NREMS returned to baseline levels for males after ZT6 (9h post deprivation).

For females, no overall significant difference was observed in NREMS during the 15h of recovery sleep following the deprivation compared to baseline (35.0 ± 8.6% vs. 47.31± 10.3%; $F(1,133)=1.996$, p=.160), however NREMS decreased significantly during the night in the first 3h post deprivation (49.2 ± 12.5% vs. 16.5 ± 8.4%, $F(1,25)=13.047$, p=.001; Figure 3.2B). Further analysis during the day following the night deprivation (ZT1-ZT12) revealed there was a significant increase in NREMS at ZT9 and ZT10 ($F(1,28)=4.443$, p=.044 and $F(1,28)=4.821$, p=.037, respectively).

REMS was suppressed following deprivation across the recovery period in both sexes, ($F(3,282)=28.706$, p<.001, data not shown). A significant interaction of treatment and sex was also observed ($F(3,282)=63.996$, p<.001). While both sexes demonstrated a significant decrease in REMS (no REMS was observed in either sex post deprivation), the relative decrease was longer in male degus, who produce more REMS than females under baseline conditions.

Additionally, there was a significant increase in NREMS bout length for male degus across the first three recovery hours following 6h of sleep deprivation during mid-night ($F_{(1,36)}$= 4.794, p=0.035; Figure 3.2C). For females, NREMS bout duration significantly increased after a prolonged delay, during the end of the day following night sleep deprivation ($F_{(1,44)}$=5.302, p=.026 and $F_{(1,44)}$=4.999, p=.031, respectively; Figure 3.2D). Thus, sleep was more consolidated for three hours immediately following deprivation and subsequently returned to baseline levels in night-deprived males, while females demonstrated a decrease in sleep bouts immediately after deprivation and a delayed increase of sleep consolidation during the latter half of the following day.

NREMS delta power during recovery demonstrated sexually dimorphic patterns that differed inversely from baseline conditions. A two-way between subjects ANOVA demonstrated there was no main effect of sex ($F_{(1,221)}$=.349, p=.555) or treatment ($F_{(1,221)}$=1.697, p=.194) during the first 15h of recovery. However, there was a significant interaction of sex and treatment during recovery ($F_{(1,221)}$=4.763, p=.030). Further analysis highlighted the increase of delta power for males over the first 3h of recovery ($F_{(1,19)}$=4.480, p=.048), yet the effect was highly transient as delta power was not significantly increased over the entire 15h of recovery ($F_{(1,103)}$=.439, p=.509; Figure 3.2E). In contrast, female degus demonstrated increased delta power not only over the first 3h post deprivation ($F_{(1,22)}$=12.859, p=.002), but also throughout the day following the night deprivation ($F_{(1,118)}$=5.598, p=.020; Figure 3.2F).

Discussion

The sleep deprivation experiments further support that the degu sleep pattern is typical of a diurnal species, largely due to the greater rebound exhibited after the night sleep deprivation compared to the day. Immediately following the day deprivation, there were few significant changes in sleep and the heightened activity at dusk was maintained. In contrast, following the night deprivation, there was a significant increase in NREMS for males and females alike, although the time course for recovery differed substantially between sexes. For male degus, there was a delay in the typical increased activity at dawn, followed by an extended morning "nap". Conversely, for females, NREMS was minimally impacted immediately after the delay and did not exhibit a significant increase until the following day. REMS was entirely suppressed for both sexes following the night sleep deprivation protocol, while not significantly altered following the day protocol in males. Additionally, there was an extended increase in NREMS delta power after the night sleep deprivation for females, while the delta power levels of males increased little following either sleep deprivation condition.

The sleep deprivation paradigm was utilized to examine the homeostatic process of these animals. The homeostatic response (significantly increased NREMS) was more pronounced following the night than the daytime deprivation within the males. NREMS bout length was increased following the night sleep deprivation, which is consistent with previous rat studies that demonstrated increased sleep consolidation because of daytime sleep deprivation (Tobler &

Borbely, 1986). In nocturnal rodents, the recovery is a direct result of the amount of increased homeostatic pressure (Tobler & Borbely, 1986).

Degus differed from these findings, because despite increased homeostatic pressure, the heightened activity periods during the light/dark transitions were preserved following both deprivation protocols in male animals and the night sleep deprivation in females. Sleep recovery was a direct result of increased homeostatic pressure for male degus, but was also strongly mediated by crepuscular activity. Following the night deprivation, there was a delay of this arousal phenomenon, yet it was still observed, highlighting a distinct difference from their nocturnal rodent counterparts.

Interestingly, following the night sleep deprivation, female degus exhibited lower amounts of NREMS during the transition compared to their baseline levels. This indicates there may be a possible weaker circadian regulation of sleep or a stronger homeostatic component for females. Baseline sleep recordings highlight both ideas as possibilities. The prolonged period of increased delta power may be indicative of increased homeostatic drive and the higher amount of sleep during the light transition may reveal weakened circadian influence on sleep. The interaction of both components of sleep may indicate a more dynamic relationship in degus, as compared to the nocturnal rat.

In degus, the heightened level of activity during the dusk/dawn periods is particularly interesting as it may be a species-specific phenomenon, and is well preserved under all conditions. This increase of activity is likely modulated by the SCN and suggests that the arousal mechanism may be particularly powerful

during these transitions, as previous research has demonstrated the SCN modulates the circadian drive for sleep and the heightened activity is likely a part of this process (Edgar et al., 1993; Easton et al., 2004). The most widely accepted sleep model posits there is a trade-off between the homeostatic and circadian components of sleep (Borbely, 1982; Achermann, 2004). These data from degus may indicate that the circadian processes during these light transition periods inhibit homeostatic mechanisms of sleep, regardless of mild increased homeostatic pressure, although this is less marked following the night sleep deprivation. These data support the hypothesis of bimodal "wake maintenance" and "sleep onset" zones that are also evident in human data (Strogatz et al., 1987), and further illustrates how degus may serve as a good diurnal animal model for sleep experiments. In order to directly investigate the circadian component of sleep in degus, further studies need to be completed under constant conditions, which was not included in the scope of this study.

The complete suppression of REMS within both sexes following sleep deprivation was not entirely surprising considering their low levels under baseline conditions (Perryman et al., Chapter 2). This effect was likely more pronounced in males because they exhibit significantly higher REMS levels during baseline conditions. Additionally, REMS rebound may have occurred after our recording period ended (48h). However, this is unlikely because previous research has illustrated the relatively transient effects of both 3h and 6h deprivation protocols (Tobler & Borbely, 1990). Higher levels of sleep deprivation may be necessary to produce a strong REMS recovery rebound. Additionally, recent research

suggests the possibility of different mechanisms for REMS homeostasis during baseline and sleep deprivation recovery conditions (Deurveilher et al., 2009). This should be an area of further exploration for both male and female degus due to their unique REMS patterns.

The different recovery timecourse of NREMS amount, consolidation and intensity between sexes is particularly interesting. Males demonstrated increased NREMS levels immediately following deprivation for both conditions, with the day deprivation inducing more highly transient changes than the night. Female degus demonstrate a significantly decreased NREMS amount immediately after night deprivation and only increase briefly during the following day. In contrast, mice showed no gender specific rebound in sleep (Koehl et al., 2006), which differed from rats, which displayed increased NREMS for females (Andersen et al., 2008).

Male degus displayed an immediate and transient increase in NREMS consolidation during the last three hours of the dark phase and subsequently returned to baseline levels during the following day. However, with females, the increase in NREMS consolidation is highly delayed and transient, appearing well into the following day. Differences in baseline sleep consolidation for males and females may account for this and appears likely based on our previous study (Perryman et al., Chapter 2). These data highlight the strength of the sex differences even under increased homeostatic drive conditions. These differences may be linked with sex differences in sleep mechanisms. The delta power data appear to support this assertion. Sleep intensity largely does not

change for males under either treatment paradigm. These results differ

dramatically from female degus. The depth of NREMS (indicated by delta power)

markedly increased during recovery for an extended period. This significant

increase of delta power suggests a sexually dimorphic compensatory mechanism

for NREMS loss in degus. Females appear to have a decreased drive for

increased NREMS amount, instead utilizing "deeper" NREMS to adapt to sleep

loss. Human and rat sleep data support this conclusion, with healthy females

demonstrating higher levels of NREMS delta power than their male counterparts,

adapting to sleep loss by increasing sleep intensity (Armitage & Hoffmann, 2001;

Andersen et al., 2008). Additionally, while males return to pre-deprivation levels

after one night of recovery sleep, an identical amount of sleep was insufficient to

reverse all sleep deprivation deficits in women (Corsi-Cabrera et al., 2003).

Some additional possibilities for females exhibiting higher delta power

levels in response to sleep deprivation may also be associated with differences in

hormone release following sleep deprivation. The stress hormones cortisol and

CRH have been demonstrated to decrease sleep in females (Steiger, 2003).

While the aforementioned experiments have utilized the least stressful protocol

for sleep deprivation, some stress is inherent to sleep deprivation. Females tend

to be more reactive to stressful stimuli than males (Herman & Cullinan, 1997;

Anisman & Merali, 1999), and increased cortisol release in response to the sleep

deprivation protocol may serve as a possible reason for the sexually dimorphic

results, particularly in the first 3h post sleep deprivation. Additionally, because

females demonstrate significantly higher NREMS levels in baseline conditions,

this could signify females reach NREMS saturation levels in recovery. Hence, delta power could serve a secondary compensatory mechanism for lost sleep.

This novel study was the first to examine sex differences in sleep following increased homeostatic pressure in a diurnal rodent. *Octodon degus* were ideal for investigating these sleep processes for a variety of reasons. Degus have well-characterized circadian rhythms and are a social, long-lived, slow-developing rodent that may serve as a good diurnal rodent model for human sleep across the lifespan. These results support their role as a unique animal for studying underlying neurobiological mechanisms for homeostatic and circadian control of sleep in a diurnal mammal.   One implication of the present findings may be that the circadian control of sleep is more powerful than the homeostatic drive in these animals during the light transitions. It would be useful to know if this phenomenon is unique to degus. Further work utilizing ovariectomized female degus is necessary for understanding the role female hormones play in sleep regulation and deprivation recovery. Additionally, longer sleep deprivation protocols during different circadian phases are required to further investigate the interaction of the circadian and homeostatic regulation of sleep for comparison to previously published data in other species. Future studies implementing stronger homeostatic deprivation or examining sleep under constant conditions may provide further insight into how these processes interact in a diurnal rodent.

Acknowledgements

Figure 3.1

Figure 3.1 A) NREMS plotted for 15h of recovery, beginning immediately

following the mid-day deprivation. Values represent the mean ±SEM. Animals

served as their own controls. There was a significant increase in NREM during

ZT14-ZT17 (p=0.03). B) There were no significant differences in NREMS bout

length at any time points (plotted for 15 h of recovery beginning immediately after

the mid-night deprivation).   C) There were no significant differences in NREMS

relative delta power observed during recovery from the sleep deprivation (plotted

for 15h of recovery immediately following the mid-day deprivation).

Figure 3.1
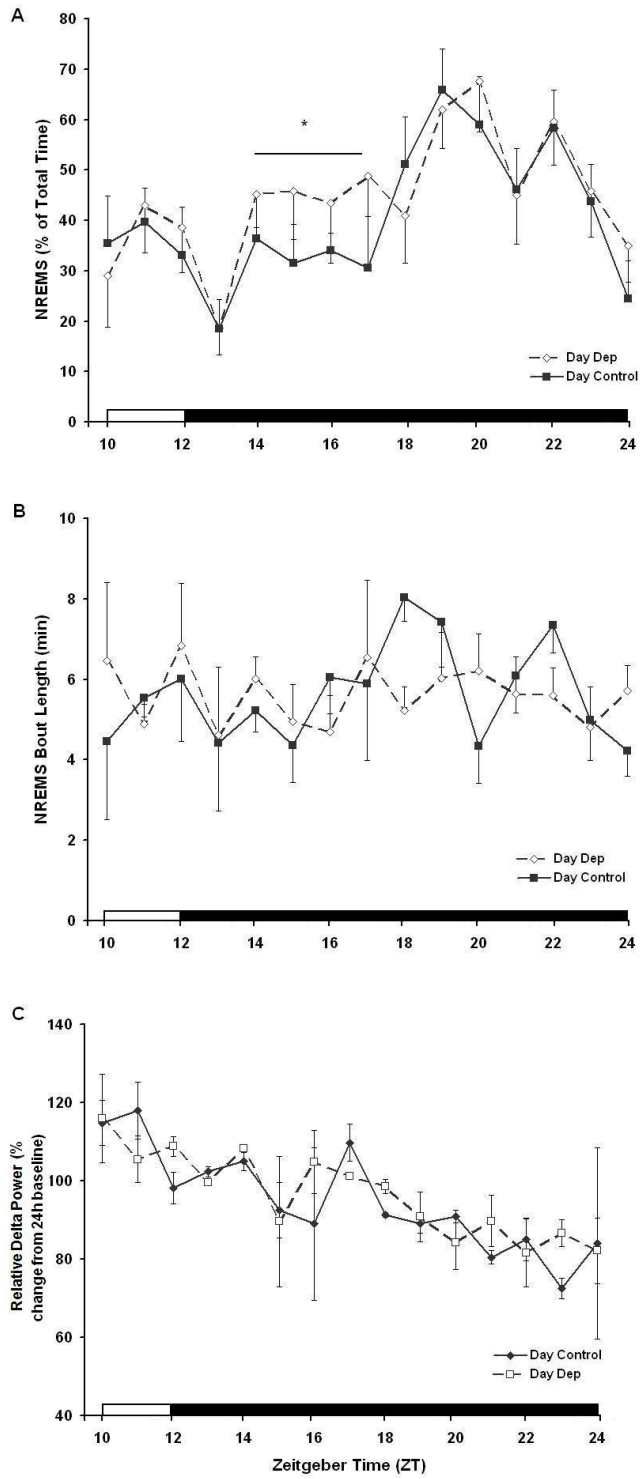
Figure 3.2

Figure 3.2 NREMS plotted for 15 h, beginning immediately after the deprivation (ZT16-ZT21) for males (A,C,E) and females (B,D,F). Values represent the mean ±SEM. Animals served as their own controls. A) In males, there was a trend of interaction between time and treatment (deprivation versus control) during the first three hours post-deprivation ($p < 0.07$) and a delay of peak activity during transition.   Additionally, there was significant increase in NREMS during ZT2-ZT5 ($p < 0.05$). B) NREMS plotted for 15 h, beginning immediately after the 6h mid-night deprivation for females. Females exhibited a significant reduction in NREMS across the first 4h of recovery ($p=.001$) and during ZT5-ZT7 ($p<0.05$), and there was an increase observed at ZT9-ZT10 ($p<0.05$). C) There was a significant increase in NREMS bout length during the first three recovery hours ($p<0.05$) in males. D) In females, there was significantly decreased NREMS boutlength during the first 4h of recovery ($p<0.05$), followed by increased boutlength during ZT4 ($p<0.05$), ZT7 ($p<0.05$), and ZT9 ($p<0.05$). E) Males demonstrated increased NREMS delta power for the first 3 hours of recovery ($p<0.05$) and during ZT2 ($p<0.05$). NREMS delta power was significantly decreased during ZT8-ZT11 ($p<0.05$) for males. F) Females exhibited higher NREMS delta power for the first 3 hours of recovery ($p=0.02$) and throughout the following day ($p= 0.002$).

Figure 3.2
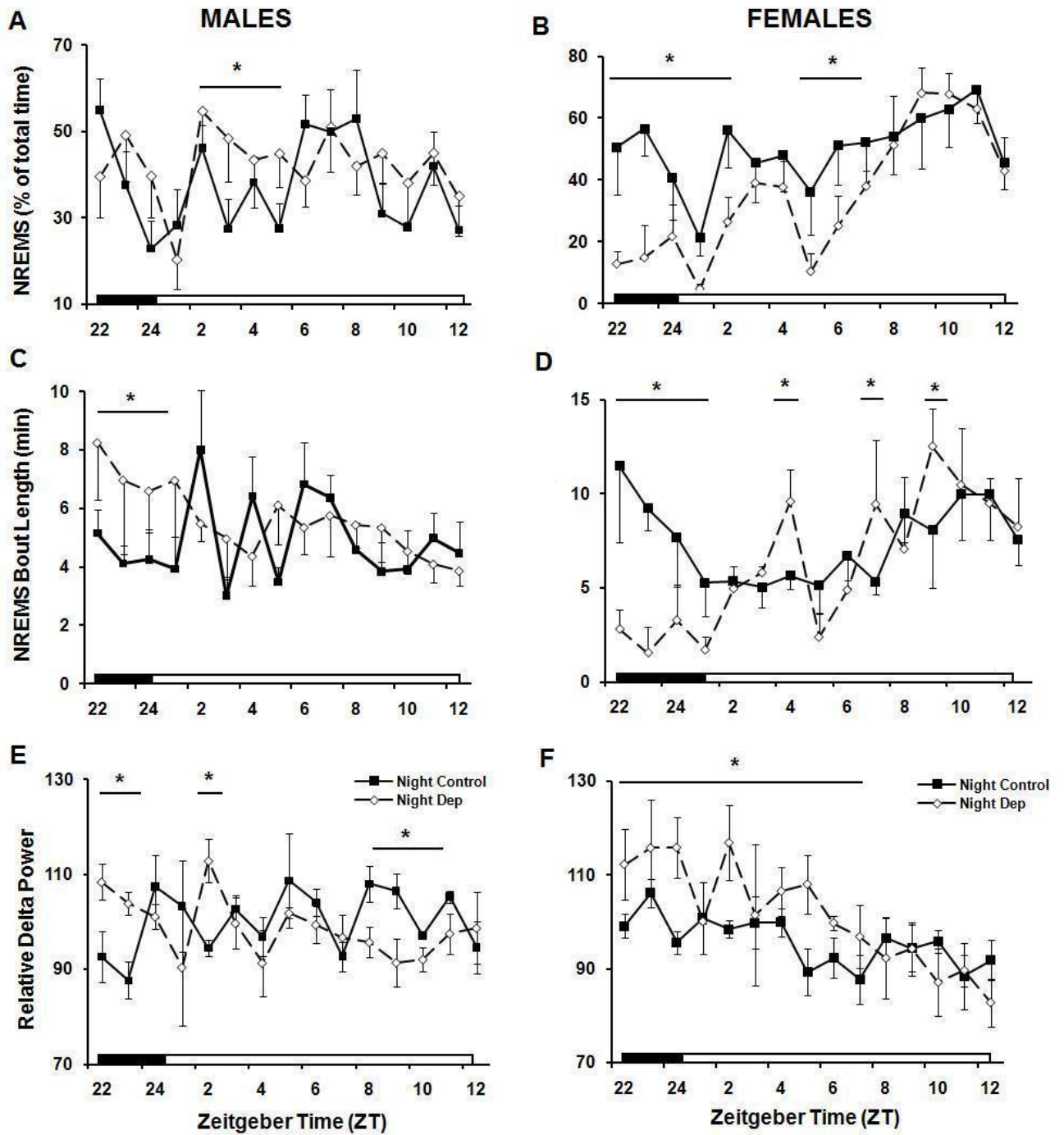
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Chapter IV
Sleep responses following increased homeostatic pressure during development in a male diurnal rodent, *Octodon degus*

Introduction

Sleep has an integral role in development and changes robustly over the course of the lifespan of many organisms. During infancy, sleep is paramount for normal brain development, with young animals spending a majority of time in this behavioral state (Feinberg et al., 1990; Miyamoto & Hensch, 2003). Sleep is divided into NREMS and REMS, and REMS predominates during early development in humans, with as much as three-quarters of sleep being devoted to this behavioral state (Frank & Heller, 1997). REMS is hypothesized to be a mechanism for synaptic plasticity, as development gives rise to the rapid formation of synaptic connections and REMS has been associated with synaptic plasticity (Bennington & Frank, 2003; Miyamoto & Hensch, 2003). Additionally, within NREMS of young animals, slow wave activity (SWA also known as delta power), which is an indicator of sleep intensity, is elevated and subsequently

declines with aging (Feinberg et al., 1990). In addition, most sleep parameters, such as sleep amount, depth and consolidation peak in adolescence and young adulthood, and decline across the lifespan (Carskadon et al., 2004).

While sleep changes across the lifespan, the most dramatic alterations in sleep architecture and timing occur during puberty, a developmental period marked by rapid brain re-organization and sexual maturation (Morishita et al., 1974; Anderson et al., 1981). Numerous studies provide evidence that the homeostatic and circadian regulation of sleep are responsive to changing gonadal hormones, especially during puberty (Leibenluft, 1993; Manber & Armitage, 1999; Carskadon et al., 2004). An influx of hormones initiate organizational (permanent) and activational (transient) effects during puberty. Previous neuroanatomical work in rats has demonstrated hypothalamic nuclei (i.e. the SCN) change in size during puberty (Morishita et al., 1974; Anderson et al., 1981), and castration eliminates the pubertal growth of these nuclei. Additionally, Branchey and colleagues (1973) demonstrate neonatal castration increased REMS in adult mice, while adult castration did not elicit any changes in sleep-wake patterns (Paul et al., 2006). Neonatal administration of exogenous testosterone reversed this phenomenon in castrated adult male mice and decreased REMS in female neonatal mice, highlighting the organizational effects hormones exert on sleep architecture.

In addition to the hormone-mediated changes in sleep amount, a delay of the circadian timing is an activational change that occurs during puberty (Carskadon et al., 1993; Crowley et al., 2007). Human sleep research suggests

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that gonadal hormones are required for the development of later sleep time during adolescence (Carskadon et al., 2008; Roenneberg et al., 2004). This delayed sleep onset correlates with development of secondary sex characteristics (Carskadon et al., 1993; Carskadon et al., 1997). Delayed circadian timing in rodents under both constant and entrained conditions is also mediated by hormones, as evidenced by elimination of the changes in circadian timing when gonadectomy precedes puberty. For example, early pubertal castration eliminated changes in circadian timing around puberty in degus (Hagenauer et al., 2009). Conversely, gonadectomized rats demonstrated a delay in circadian timing following gonadectomy, but the amplitude of the delay was smaller than found for intact rats. This suggests a partial role for gonadal hormones in producing developmental changes in circadian timing for rats (Hagenauer et al., 2009). The differential regulation of developmental circadian timing between nocturnal and diurnal rodents underscores the need for further experiments examining circadian and sleep parameters during development across a variety of species.

In humans, a theoretical model of this altered circadian phase was developed, which accounted for changes of the homeostatic and circadian components of sleep that accompany puberty (Carskadon, 2008). Current research theorizes that homeostatic drive for sleep increases with the duration of waking and dissipates during sleep, while the circadian system alternately promotes wakefulness and sleep, depending on the phase (Achermann & Borbely, 2003). Researchers posit human adolescents develop resistance to

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sleep pressure, while circadian phase becomes delayed concurrently

(Carskadon, 2008).   In support of this theory, early pubertal adolescents appear

to have equal dissipation rates of sleep pressure across the night, however the

buildup of delta power following sleep deprivation was slower in post-pubertal

compared to pre-pubertal children (Jenni et al., 2005a; Jenni et al., 2005b).

Within rodents, sleep deprived juvenile rats exhibit more NREMS,

increased consolidation of sleep episodes and higher delta power as compared

to their own baseline and compared to adults (Trachsel et al., 1986; Alfoldi et al.,

1990). This heightened response diminished across puberty, and by late

puberty, young rats exhibited decreased total amount of NREMS and delta power

in baseline conditions and a dampened response to sleep deprivation compared

to a younger age (Alfoldi et al., 1990). These experiments highlight a potentially

increased responsiveness of adolescent sleep to disruption and represents a

largely unexplored area of research. Degus are ideal for investigating the

aforementioned topics because they are a slow developing diurnal rodent whose

circadian rhythms, developmental time course and sexual maturation have been

well studied and clearly characterized (Jechura et al., 2000; Lee et al., 2004;

Hummer et al., 2007).

Within my study, I aimed to characterize the baseline sleep of intact male

early-pubertal (three-month-olds) and late-pubertal degus (six-month-olds), and

investigate the response to sleep disruption across adolescence. Sleep was

quantified at critical periods in the developmental cycle (during early and late

puberty) to elucidate circadian and homeostatic components of sleep during

development for a diurnal rodent. For the first experiment, I collected seventy-two hours of baseline sleep recordings from three-month-old and six-month-old degus and examined its architecture, depth and timing. Subsequently, both age groups were subjected to sleep disruption (via 6h sleep deprivation) and allowed uninterrupted recovery sleep. I hypothesized that three-month-old degus (early pubertal) would display increased NREMS, REMS, higher sleep consolidation and delta power during baseline conditions compared to six-month-old degus (late pubertal). Additionally, I hypothesized three-month-old degus would exhibit a greater response to sleep deprivation compared to six-month-olds, revealing a greater vulnerability to sleep disruption among younger degus. Moreover, I expected both age groups to exhibit higher NREMS, REMS, sleep boutlength and delta power compared to male adult degus.

Methods

Animals and Surgery

Thirteen juvenile male *Octodon degus* aged 3mo (n=7) and 6mo (n=6) were used in this study. Degus were obtained from The University of Michigan colony. Animals were housed individually in recording chambers and maintained on a 12:12 light/dark cycle. Recording chambers [Nalgene cages (42.5 x 22 x 19cm)] were designed with a cover to accommodate a tether system that enabled collections of electroencephalography (EEG), locomotor activity rhythms (via infrared detections), and brain temperature (via brain thermistor). Food (Purina

5001 Rodent Chow) and water were provided *ad libitum*. The ambient temperature was 18 ± 1°C.

Animals were anesthetized with 5% isofluorane, positioned in a stereotaxic device and surgically implanted with screw electrodes that allowed the collection of EEG recordings. Two frontal and two occipital epidural EEG leads were placed in position using stainless steel screws (adapted from Kas & Edgar, 1999). A thermistor for brain temperature monitoring was implanted under the skull on top of the dura between the two sets of screws. All leads were connected to a pedestal autoclaved prior to surgery. The pedestal was fixed to the skull with dental acrylic after skull surface was dried and coated with Superglue. Body temperature of animals was maintained with a water-filled heating pad during surgery and post-operatively until arousal. The surgical opening was subsequently covered with Nolvasan antiseptic ointment, and animals were given 3-4cc of Lactated Ringer's Solution subcutaneously (s.c.) following surgery to prevent dehydration. Prophylactic antibiotic was given postoperatively (20mg/kg chloramphenicol, s.c.) for 4 days. Possible postoperative pain was managed with butorphinol (0.10 mg/kg intraperitoneal [i.p.] preoperatively and 0.05 mg/kg postoperatively s.c). The animals were also given a post-operative one-time injection (s.c) of dexamethasone (0.2 mg/kg) to reduce swelling post-operatively. The condition of post-surgical animals was monitored twice daily for several days and pain medication (butorphinol) was administered as needed to alleviate discomfort. The animals were fed 1 ml of Nutri-cal (a high calorie supplement) and water twice daily during the post-operative period. Degus were weighed daily and animals were

categorized as fully recovered when weight stabilized. Prior to beginning data collection, the degus spent at least one week habituating to the recording chamber, including being tethered before collecting baseline EEG data. All degus were singly housed during data collection and given at least one week of recovery between each treatment condition. Between experiments, juvenile degus were returned to paired housing to minimize possible brain and emotional deficits of prolonged isolation during the developmental period of these animals (Lewis et al., 1990). All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee.

Sleep Deprivation Experiment

Seventy-two hours of baseline sleep recordings were collected from three month old (n=7) and six month old (n=6) male degus. Subsequently, both age groups were sleep deprived by gentle handling (6h). In previous studies utilizing nocturnal rodents, sleep deprivation normally begins immediately following lights off or immediately prior to lights on. Due to their increased activity around dusk and dawn, degus were deprived during the middle of the dark phase (ZT16-22). Baseline recordings were collected immediately prior to the sleep deprivation protocol. EEG, temperature, and activity rhythms were collected for 48 h during an uninterrupted recovery sleep period. Animals served as their own controls (pre- and post-experimental data were analyzed and compared).

Sleep data analysis

The EEG was processed through an eight-channel Grass polygraph (Grass Technologies, West Warwick, RI). EEG was passed through filters (Coulbourn Instruments, LeHigh Valley, PA) and into a data acquisition system that samples, digitizes and stores the data. ICELUS, a computer-assisted visual scoring software (Mark Opp, Ann Arbor, MI), was used to categorize sleep states. Several measures were introduced into the program, including EEG, brain temperature, and locomotor activity (LMA collected using an infrared device placed atop the recording chamber) to accurately identify and designate sleep states with a 12-s resolution, which were characterized using the ICELUS program. NREMS was categorized by high amplitude, low frequency EEG signal with little or no locomotor activity. REMS was characterized by high frequency, low amplitude, theta dominated EEG signal with no locomotor activity. Wakefulness was characterized by mixed voltage, high frequency EEG signal with varying amounts of locomotor activity. Spectral analysis was also performed on the sleep data. Delta power (sleep intensity as measured by slow wave activity [SWA]) during NREMS was quantified under the 0.5Hz-4.5Hz frequency band.

Statistical Analyses

NREMS, REMS, WAKEFULNESS (WAKE), DELTAPOWER, TRANSITIONS (TRANS), number of NREMS bouts (NRBOUTS), number of REMS bouts (RBOUTS), NREM bout duration (NRBOUTLENGTH), and REM bout duration (RBOUTLENGTH) were compared between baseline and treatment conditions, as well as between age groups. Analysis of variance

(ANOVA) with post hoc *Tukey* analysis and *t tests* were used to determine

significant differences in the sleep of animals between the treatment conditions

(baseline, deprivation and phase shift) and across age. All statistical tests were

considered significant with an α<0.05.

Results

Three month old degus during baseline conditions

Baseline sleep recordings demonstrated significant differences in the

sleep of early pubertal (three month old) and late pubertal (six month old) degus.

Utilizing paired *t test* analysis, 3 month olds showed no significant differences in

NREMS (t(71)= -1.131, p=.262), REMS (t(59)=1.056, p=.295), WAKE

(t(59)=.674, p=.503), TRANS (t(71)= -.779, p=.439), and NRBOUTS (t(71)=.973,

p=.334) between the day and night (Table 1). However, NRBOUTLENGTH was

increased during the night compared to the day (t(71)=2.003, p=.049). Moreover,

DELTAPOWER demonstrated a strong rhythmicity, building over the day and

peaking during the early night and subsequently declining across the night with

repeated measures ANOVA (*F*(1,23)=2.841, p< .001; Figure 4.1E).

Six month old degus during baseline conditions

Six month old animals demonstrated a circadian variation of NREMS

(t(71)= -2.705, p=.009), REMS (t(71)=4.399, p< .001), and WAKE (t(71)= -2.956,

p=.004), showing a significant increase in these factors during the night

compared to the day (Table 1). Conversely, NRBOUTLENGTH (t(71)= -1.489,

p=.139), TRANS (t(71)= -1.810, p=.074), and NRBOUTS (t(71)=1.526, p=.132) remained relatively constant across the LD cycle. DELTAPOWER lacked clear rhythmicity in the older group (F(1,23)=.758, p=.769; Figure 4.1F).

Comparisons of baseline sleep across development

Six month old degus exhibited robust differences across the day and night compared to their younger counterparts. During the dark period, six month old animals exhibit higher levels of NREMS (t(71)=3.657, p< .001) and lower levels of WAKE(t(71)= -3.171, p=.002) compared to three month old degus.

In between age groups, a significant increase of NREMS across puberty emerged (t(10)=2.377, p=.039; Figure 4.1A). Additionally, REMS was increased for three month olds compared to six month old animals (t(9)= -2.586, p=.029; Figure 4.1B). Total amount of WAKE did not change between three and six months (t(9)= -2.192, p=.056; Figure 4.1C). Furthermore, NRBOUTLENGTH (indicated by NREMS bout duration) was comparable between age groups (t(10)= -.122, p=.905; Figure 4.1D). One way between subjects ANOVA demonstrated three and six month olds DELTAPOWER did not demonstrate significantly different values (F(66,96)=1.550, p=.097). Additionally, TRANS (t(10)= -.216, p=.833) and NRBOUTS (t(10)= -.391, p=.704) were comparable between age groups, indicating sleep efficiency was not changed across puberty during baseline conditions.

Sleep Deprivation

Following baseline data collection, both groups were sleep deprived for 6h and permitted uninterrupted recovery sleep (48h) to analyze sleep rebound following increased homeostatic pressure. The first 15h of recovery is described here. Significant differences in the recovery sleep of three-month-old and six-month-old degus were exhibited compared to baseline conditions (Figure 4.2A,B). NREMS demonstrated no main effect of age over the 15h recovery ($F(1,298)=.004$, $p=.951$). There was a robust main effect of deprivation on NREMS levels ($F(1,298)=12.891$, $p<0.001$), but no interaction of deprivation and age ($F(1,254)=.004$, $p=.948$) was observed.

The increased activity around the light/dark transition for both groups of juvenile degus appeared to be delayed for one hour during baseline conditions compared to adult males degus. However, following the night deprivation, while late pubertal degus and adults (Chapter III) demonstrated an hour delay of circadian gating, early pubertal degus maintained increased activity at the same circadian phase as baseline, illustrating circadian gating is a particularly robust phenomenon during early puberty for degus compared to late pubertal and adult animals. Analysis of the early pubertal compensatory sleep response revealed NREMS returned to baseline levels for juvenile animals the following night (15h post deprivation, data not shown).

Even with the small amount of REMS in adult and juvenile degus, REMS was not suppressed following deprivation across the recovery period, as it was in adults (Chapter III). Statistical analysis highlighted the minimal impact of sleep

deprivation on REMS during recovery sleep ($F_{(1,254)}$=.213, p=.645). However, REMS was significantly impacted by age ($F_{(1,254)}$=8.912, p=.003; Figure 4.2C, D), but a significant interaction of treatment and age on REMS was not observed ($F_{(1, 254)}$=.020, p=.889). Neither group demonstrated a significant alteration in REMS (REMS was observed in both age groups post deprivation), likely due to the early pubertal degus displaying significantly higher REMS levels during baseline conditions.

Additionally, there were no significant overall changes in NREMS bout length for juvenile degus following 6h of sleep deprivation due to treatment ($F_{(1,254)}$=.012, p=.912), but age demonstrated a robust influence on NREMS bout duration, with early pubertal degus exhibiting more consolidated NREM bout duration compared to late pubertal degus ($F_{(1,254)}$=7.100, p=.008; Figure 4.2E,F).

DELTAPOWER during recovery demonstrated significant increases, especially in three month old degus. Three month old degus demonstrated increased delta power throughout the entire 15h following the night deprivation ($t_{(119)}$=7.453, p<.001; Figure 4.2G). Six month olds did not display significantly increased DELTAPOWER over the 15h recovery period, but further analysis highlighted the increase of delta power for six month olds over the first 4h of recovery ($t_{(19)}$=-5.040, p<0.01; Figure 4.2H), yet the effect was relatively transient, as DELTAPOWER was increased over 15h of recovery for three month olds. The following night of rebound sleep demonstrated there were no long

lasting effects of the 6h sleep deprivation, as both age groups returned to baseline levels of all sleep parameters (data not shown).

Discussion

These data represent the first study to investigate the adolescent homeostatic and circadian components of sleep under baseline and disrupted conditions in a diurnal rodent. During baseline conditions, early pubertal degus (three-month-olds) did not display a strong diurnal preference, but delta power and sleep consolidation were consistent with diurnality. Conversely, late pubertal degus (six-month-olds) demonstrated a diurnal preference, with clear circadian variation of NREMS and REMS, which is consistent with previous data indicating a critical timing period for hormonal impact on sleep. Both age groups exhibited high activity during the "dusk" light transition, indicating strong circadian gating in this species. Following sleep deprivation during the dark phase, both age groups demonstrated a robust increase in NREMS for an extended period, although late pubertal degus showed a more marked increase in NREMS. Additionally, delta power significantly increased across the first 15h of recovery for early pubertal degus while late pubertal degus demonstrated a brief increase in sleep intensity, which more closely followed the rebound sleep patterns of adult male degus.

Interestingly, there were no significant differences in total sleep amount, NREMS, REMS or sleep consolidation between the early and late pubertal degus under baseline conditions. Additionally, neither group differed significantly from adult levels of NREMS or REMS (see Chapter II and III). However, there were

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significant differences in delta power and sleep consolidation from baseline sleep

recordings, which supports previous findings demonstrating a decline in these

sleep parameters during development (Trachsel et al., 1986; Alfoldi et al.,1990).

Young animals exhibited significantly increased homeostatic drive to sleep

following sleep deprivation, regardless of circadian phase during the deprivation

protocol (Alfoldi et al., 1990), highlighting the powerful compensatory mechanism

for lost sleep during development. These results are consistent with the earlier

findings, as both groups displayed a strong rebound of NREMS during recovery

following the 6h deprivation. Conversely, delta power for six month olds

demonstrated a transient increase following the deprivation, while their younger

counterparts exhibited a highly robust increase in delta power across the 15h

recovery period. Both age groups demonstrated strong enhancement of sleep

throughout the day subsequent to the deprivation, highlighting the increased

responsiveness to increased homeostatic pressure conditions during this

developmental period in degus.

Interestingly, heightened activity during light transitions was delayed in

both age groups compared to adults during baseline conditions, yet following the

deprivation protocol, no delay in activity was observed in early pubertal degus.

This differs from the adult data that suggested activity during the light transitions

is more powerful than homeostatic drive to sleep in this species. Conversely,

heightened activity appeared delayed in late pubertal degus following the

deprivation paradigm, a finding that is similar to adult males (see Chapter III).

This suggests there may be an intermediate "adjustment" phase during

development. Moreover, heightened activity during the light transitions was robust in both early and late pubertal degus during baseline, suggesting heightened activity during the light transitions is not directly mediated by gonadal hormones during puberty. These findings are further supported by data illustrating that even as degus become phase delayed during puberty, activity around the light transition periods remains stable, highlighting that this circadian parameter may not be mediated by hormone levels (Hagenauer et al., 2009).

Additionally, previous electrophysiological studies in adult male degus have highlighted the strongly circadian mediated activity during "dusk", with a weaker signal occurring during "dawn" (Kas & Edgar, 1998). Human data have provided strong evidence for this evening circadian arousal signal, commonly referred to as a "wake maintenance zone" due to its oppositional role to increasing homeostatic pressure (Strogatz et al., 1987; Munch et al., 2005). Furthermore, the evening circadian arousal signal has been demonstrated to decline with age (Munch et al., 2005), which may provide insight into the stark contrast of activity between juvenile and adult male degus. This suggests homeostatic and circadian sleep processes are modulated during development and mediated differentially under baseline and deprivation conditions for adolescents.

Conversely, the low amount of REMS in juvenile degus during baseline and sleep deprived conditions was unexpected and may indicate a unique function for REMS within this species. Testosterone and estrogen have been demonstrated to reduce REMS in rats and mice (Branchey et al., 1973; Fang &

Fishbein, 1996; Paul et al., 2006), yet three month old degus demonstrated comparable levels of REMS with adult males, which implies the low amount is not regulated by hormones. Therefore, the consistently low REMS levels across age groups may be a result of the overall low levels of REMS within this particular species. While REMS has been associated with synaptic plasticity, there is growing evidence of synaptic plasticity associated with delta power levels in NREMS as well (Tononi & Cirelli, 2006, Massimini et al., 2007; Massimini et al., 2009). Low levels of REMS may indicate that degus utilize more delta power mediated synaptic plasticity. Interestingly, while degus exhibit low levels of REMS that are relatively constant throughout development and young adulthood, following sleep deprivation, both groups of juvenile degus failed to demonstrate a suppression of REMS and exhibited REMS rebound during recovery.   This is in stark contrast to sleep deprived adult degus of both sexes, which exhibited suppression of REMS and lack of REMS rebound during recovery sleep. This suggests REMS is essential for young degus despite of the low overall amount. Additionally, researchers have asserted that REMS may demonstrate different homeostatic mechanisms for baseline and recovery conditions (Deurveilher et al. 2009) and the degu juvenile data is consistent with this hypothesis. Further work is necessary to elucidate REMS homeostasis under various conditions and across species.

A sleep parameter that is largely consistent across development of many species is sleep consolidation, which during early life is prominent and becomes more fragmented with aging (Trachsel et al. 1986; Tobler &Borbely 1990).   Our

data support this hypothesis, with both groups of juvenile degus demonstrating substantially longer NREMS bout duration compared to their adult male counterparts (see Chapter II). The reduction of sleep consolidation with age has been hypothesized to be partially mediated by a loss of VLPO neurons, which has been widely accepted as a sleep promoting nuclei, yet neuronal loss in the VLPO has not been definitively linked with sleep loss due to aging (Shiromani et al., 2000; Gaus et al., 2002; Saper et al., 2005). The literature suggests a more likely candidate for a prominent role in regulation of sleep consolidation is the suprachiasmatic nucleus (Barakat et al., 2004), as lesion studies have demonstrated sleep becomes arrhythmic and fragmented following a loss of neurons within the SCN (Czeisler et al., 1980; Ibuka et al., 1980; Edgar et al., 1993; Larkin et al., 2004). Since previous studies have illustrated anatomical and physiological changes in the SCN during puberty (Morishita et al., 1974; Morishita et al., 1978; Anderson et al., 1981), this may account for the marked decline in sleep consolidation with aging. Additionally, human research has indicated sleep becomes more vulnerable to stress hormone effects (i.e. cortisol) beginning in adulthood (Van Cauter et al., 2000; Kalus et al., 2009), which may mediate lower consolidation levels in adults.

As expected, baseline delta power demonstrated a robust rhythm in juvenile degus, consistent with models of homeostatic drive within diurnal species (Achermann & Borbely, 2003). A robust increase in delta power after the deprivation was evident in the juvenile data and this elevation continued throughout the following day. Increased sleep intensity due to prior sleep

deprivation has been demonstrated to be more robust in juvenile animals compared to adults (Trachsel et al., 1986; Alfoldi et al., 1990). This decline of delta power across the lifespan has been well characterized and is the source of much speculation. Feinberg (1990) demonstrated cortical synaptic density decreases across the lifespan, and delta power follows the time course of this decline, indicating delta power may be mediated by synaptic strength. Tononi and Cirelli (2003) posited that the function of delta power is to mediate synaptic homeostasis and past findings are consistent with this emerging theory, yet clear causal evidence is still lacking.

There is a dearth of literature examining sleep and development within diurnal mammals (humans not withstanding). This novel work was the first to investigate baseline sleep in juvenile diurnal rodents, as well as the response to sleep deprivation across development. Degus were utilized for these experiments because this species is a social, long-lived, slow developing species which may serve to elucidate changes in sleep across the lifespan. These results support this hypothesis, with degus displaying dynamic changes of the circadian and homeostatic sleep components with development. Future work utilizing gonadectomized males during development is necessary to elucidate the precise influence of pubertal gonadal hormones on sleep regulation. This work would further illuminate the hormonal influence on sleep during development, and may provide insight into whether there is a critical time period of organizational sleep effects in male degus, as these data and other studies suggest. Additionally, the sleep of intact and ovariectomized female degus

should be explored across development to accurately characterize the sleep

processes between sexes, as well as in response to physical and physiological

disruption.

Acknowledgements

Table 4.1: Baseline sleep architecture for early pubertal (three-month-old) and late pubertal (six-month-old) male degus

| Sleep Parameter by Age	Light period	Dark period	Total 24h
Wake duration (% of recording time)			
Three Month Olds	62.96 ± 1.98	60.22 ± 3.01*	61.6 ± 6.6
Six Month Olds	61.41 ± 2.33	50.14 ± 2.68†	55.8 ± 6.8
NREMS duration (% of recording time)			
Three Month Olds	34.86 ± 1.66	38.63 ± 2.44*	36.7 ± 1.3*
Six Month Olds	37.76 ± 2.23	47.39 ± 2.47†	42.6 ± 2.1
NREMS bouts (number/h)			
Three Month Olds	4.28 ± 0.28	3.86 ± 0.26	4.1 ± 0.8
Six Month Olds	3.59 ± 0.26	4.12 ± 0.23	3.9 ± 0.8
NREMS bout duration (min)			
Three Month Olds	5.23 ± 0.32	6.55 ± 0.53†	5.9 ± 1.3
Six Month Olds	5.52 ± 0.32	6.15 ± 0.32	5.8 ± 1.0
REMS duration (% of recording time)			
Three Month Olds	1.99 ± 0.26*	2.51 ± 0.36	2.2 ± 0.2*
Six Month Olds	0.83 ± 0.16	2.47 ± 0.31†	1.6 ± 0.6
Transitions (number/h)			
Three Month Olds	8.68 ± 0.58	7.99 ± 0.55	8.3 ± 0.6
Six Month Olds	7.42 ± 0.54	8.79 ± 0.53	8.1 ± 0.9

*=Significant difference between age groups ($p < 0.05$)
†=Significant difference within an age group ($p < 0.05$)

Figure Caption 4.1

Baseline sleep recordings demonstrated significant differences in the sleep of three month old and six month old degus. Values represent the mean ± SEM. Black closed diamonds denote three month old degus and open squares represent six month old degus. A) Three month old degus did not demonstrate significant circadian variation in NREMS, while six month old degus displayed a diurnal sleep rhythm across the baseline recordings (p< 0.05). Six month old animals exhibited significantly increased NREMS compared to their younger counterparts. B) REMS for both groups across baseline is plotted. Six month old degus demonstrated significantly less REMS than three month olds, but exhibited more REMS during the night compared to the day (p<0.05). Three month olds revealed no circadian difference in REMS. C) Six month olds demonstrated significantly less WAKE during the night compared to the three month olds and their own daytime values (p<0.05). Three month olds demonstrated no significant circadian variation in WAKE across the baseline recording period (p>0.05). D) Six month old degus did not demonstrate significant differences in NREMS boutlength compared to their younger counterparts (p>0.05). E) Relative delta power of three month olds demonstrated a significant rhythm across baseline, increasing over the day, peaking early during the night and dissipating across the night (p=. F) Baseline relative delta power of six month olds displayed a flattened rhythm across the 24h recording period.

Figure 4.1
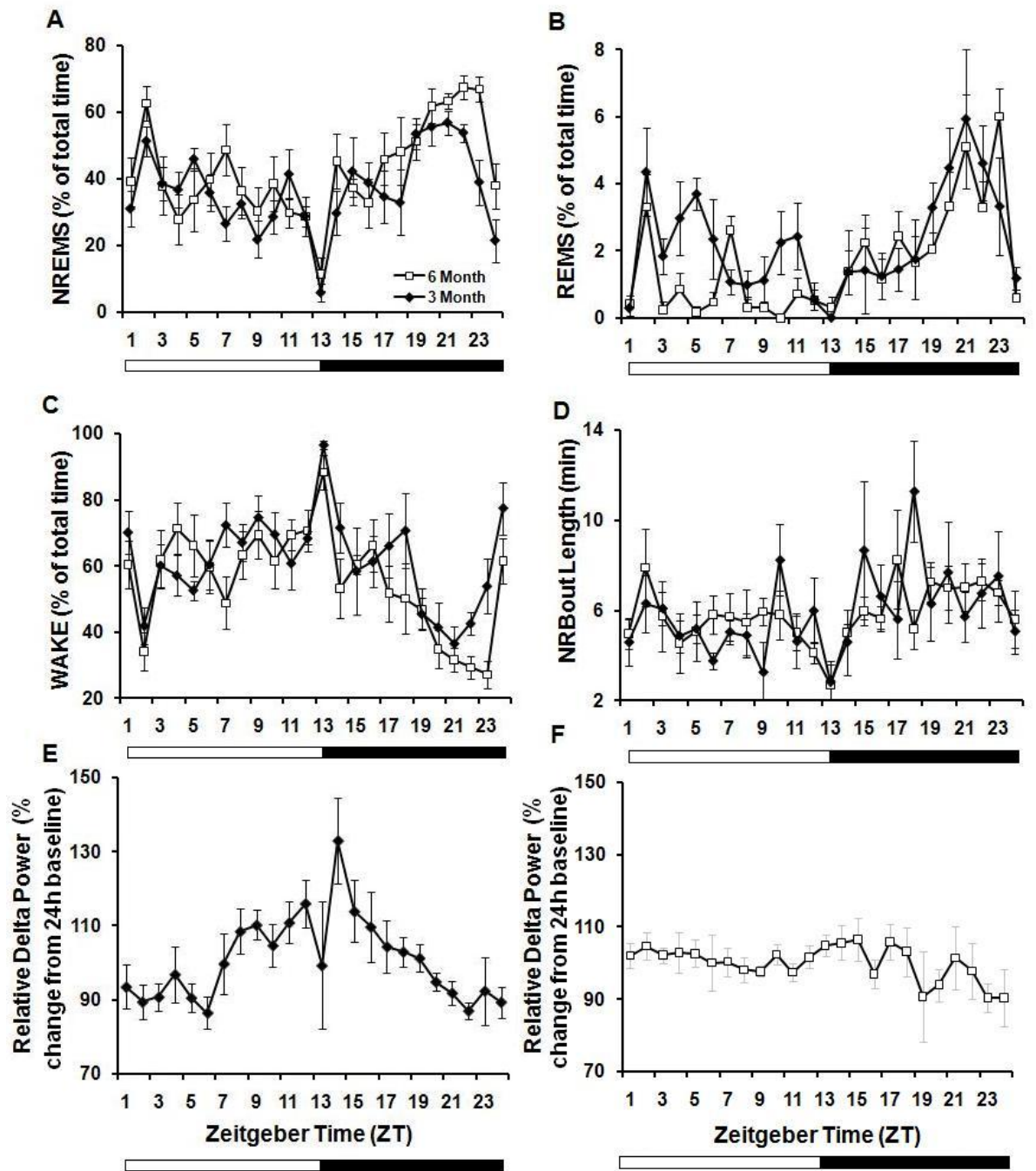
Figure Caption 4.2

Sleep parameters of pre-pubertal (A,C,E) and pubertal degus (B, D, F) plotted for 15h of recovery, beginning immediately following a 6h deprivation during the dark phase. A) NREMS following the 6h night deprivation protocol. Values represent the mean ± SEM. Animals served as their own controls. There was a significant increase in NREMS across the 15h recovery period for pre-pubertal degus ($p<0.05$). B) NREMS during 15h recovery following a 6h night deprivation. For pubertal degus, there was a significant increase in NREMS across the entire 15h recovery period ($p<0.05$). C) REMS following the 6h night deprivation protocol. No significant differences in REMS were observed across the recovery period for three-month-olds. D) REMS immediately following the 6h night deprivation. No significant differences in REMS were observed across the recovery period for pubertal degus. E) NREMS bout duration following the 6h night deprivation protocol. Early pubertal animals demonstrate relatively unchanged sleep consolidation during recovery. F) NREMS bout duration following the 6h night deprivation. Late pubertal degus exhibited increased NREMS consolidation briefly immediately following the deprivation. G) Relative delta power following the 6h night deprivation protocol. Early pubertal degus displayed a robust increase in delta power during recovery ($p<.001$). H) Recovery delta power following the 6h night deprivation protocol. Six-month-olds exhibited a low amplitude, transient increase in delta power during ZT22-ZT2 ($p<0.05$).

Figure 4.2
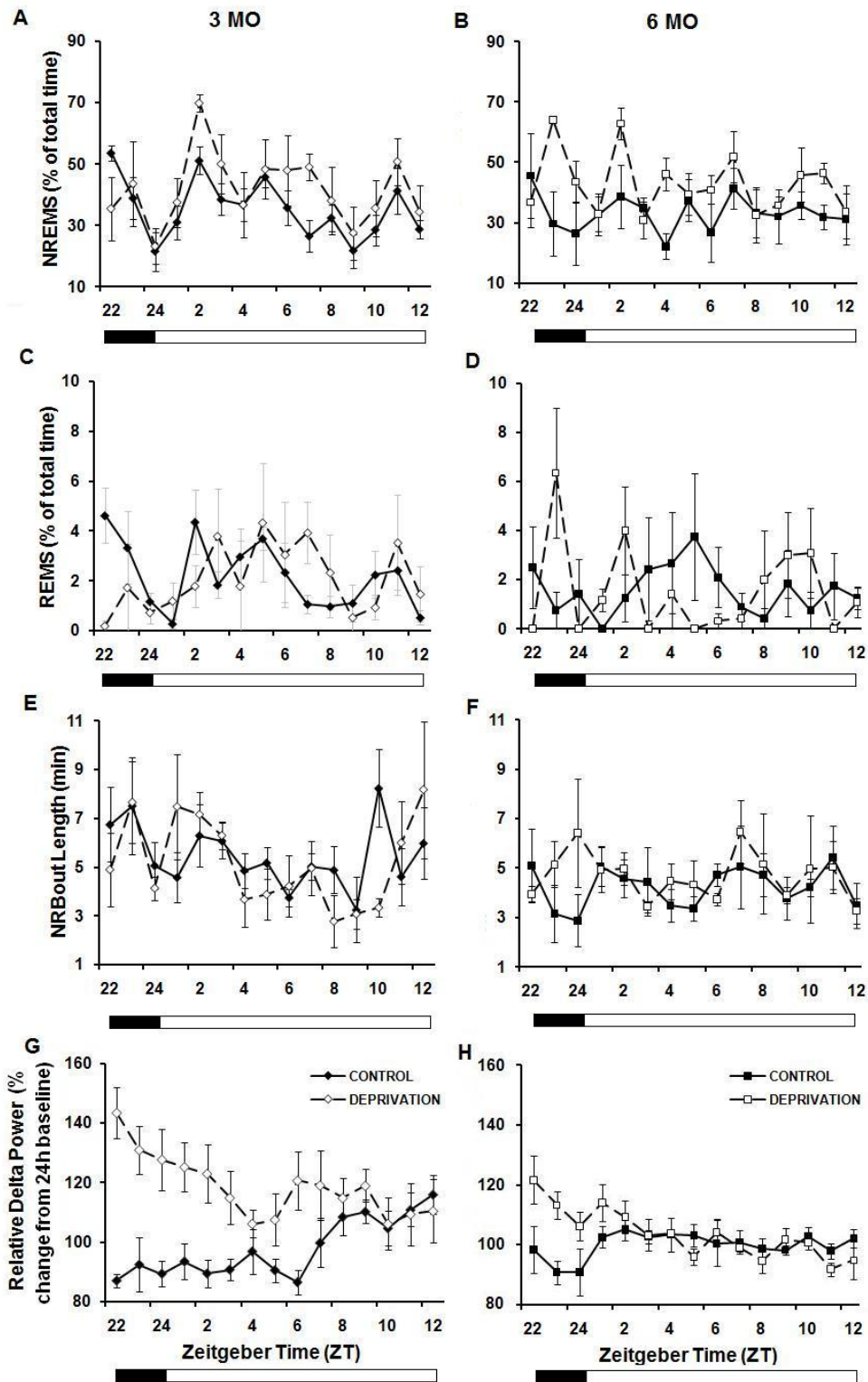
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Chapter V
Perspectives on Sleep in a Diurnal Rodent

Summary of Findings

Chapters two through four examined how sex and development impacts sleep. My initial descriptive study revealed the diurnal sleep patterns of degus and the low levels of REMS, with females showing significantly more NREMS amount and consolidation and less REMS compared to males. SWA was sexually dimorphic, with males demonstrating higher relative levels during the light phase and females exhibiting increased SWA during the dark phase. Circadian gating was particularly powerful, with both sexes displaying heightened activity around the light transitions. My second set of experiments aimed to elucidate the homeostatic and circadian components of sleep utilizing the sleep deprivation paradigm. Sleep deprivation increased homeostatic drive within both sexes and was mediated by circadian phase. Compensatory mechanisms for recovery of lost sleep differed between sexes, with males demonstrating transient increases in NREMS amount and consolidation while females displayed transitory increases in NREMS consolidation and prolonged elevation of sleep

intensity. Lastly, I investigated the two components of sleep across development within male degus. My experiments highlighted the high levels of overall sleep within adolescent degus compared to adult males. While three month old degus did not demonstrate a strong preference for diurnal sleep, the rhythmicity of delta power was consistent with previous studies of diurnal mammals. Six month old degus demonstrated circadian variation of both NREMS and REMS (in contrast to adult REMS), suggesting there may be a critical window of hormonal influence on sleep within this diurnal rodent. Whether these changes are due to hormones or simply the aging brain merits further investigation. Both age groups displayed significantly increased NREMS, consolidation and sleep intensity in response to a 6h deprivation during the middle of the dark phase. Additionally, the highly conserved circadian light transition activity bouts of the adult degus are greatly reduced in young animals following the deprivation protocol. Together, these data highlight *Octodon degus* as a good diurnal rodent model for investigating the circadian and homeostatic mechanisms of sleep, as well as the interaction of these opponent processes under baseline conditions and following physiological disruption. Furthermore, the slowly developing degu presents a unique opportunity to examine these components in a readily accessible small diurnal mammal across the lifespan and between sexes.

Sleep, Sex and Hormones

The stark contrast of NREMS distribution between sexes was particularly interesting. The high levels of NREMS and low levels of delta power during

baseline recordings for female degus during the light phase were unexpected.

For female degus, this may indicate a divergent mechanism for sleep efficiency

or a lingering negative rebound following a dark phase ceiling effect of

homeostatic drive. Further, this may highlight decreased efficiency of daytime

sleep within female degus compared to males. Female sleep during the day may

be lighter and less restorative, which leads to increased need to maintain

homeostasis. Further analysis of NREMS during the light phase may reveal the

exact architecture. Perhaps more light sleep (Stage 1 & 2) is exhibited during

this period, which may account for the increased NREMS amount and decreased

SWA for females. I may be able to examine this by quantifying spectral data

within the sleep spindle frequency, which typically is used to quantify light sleep.

Since NREMS is not differentiated into distinct stages (S1-S2 "light sleep" & S3-

S4 "deep slow wave sleep") in most rodent studies, this may provide an indirect

way to investigate the specific characteristics of daytime NREMS in female

degus.

In addition, sex differences in REMS expression were very apparent, with

male degus demonstrating twice the amount of REMS during baseline compared

to their female counterparts.   Sex hormones are suggested to play a large role in

sleep regulation and my research supports this hypothesis. Progesterone is

reported to suppress REMS, decrease wakefulness and shorten latency to

NREMS (Gandolfo et al., 1994; Lancel et al., 1996), while estrogen increases

norepinephrine within sleep promoting brain areas like the brainstem,

hypothalamus, locus coeruleus, which enables noradrenergic neurons to

suppress REMS in rats (Fang & Fishbein, 1996). Researchers have postulated systemic effects of hormones produce these changes, but previous work offers little evidence supporting this view as circulating hormones have very little impact on sleep in adulthood (Branchey et al., 1973; Fang & Fishbein, 1996).

Furthermore, sleep may be mediated by levels of hormone receptors in sleep promoting brain areas rather than amounts of circulating hormones. Previous work has revealed hormone receptor levels in sleep promoting (i.e. VLPO) and arousal nuclei areas (i.e. tuberomammillary nucleus and dorsal raphe) may be vital to understanding the sex differences in sleep (Shechter & Boivin, 2010). Research has highlighted the importance of the VLPO for NREMS, as lesions of this area produce insomnia (Gaus et al., 2002). In addition, the extended VLPO plays an integral role in REMS (Chou et al., 2002; Lu et al., 2002). Since both testosterone and estrogen have been shown to suppress REMS, the decreased amount of REMS in female degus may be mediated by differing levels of hormone receptors within the extended VLPO or decreased sensitivity of receptors within this area. Additionally, prostaglandin receptors within the VLPO are modulated by estradiol, and may represent a mechanism for the lower REMS levels exhibited by females (Qu et al., 2006). Moreover, the raphe nuclei, which contain hormone receptors, project directly to the pontine nuclei involved in REMS generation (McCarley, 2004; Fuller et al., 2006). Conversely, variations in the density of the serotonergic projections to the brainstem nuclei may also mediate REMS amount without hormonal input.

Following the 6h deprivation protocol, males appeared to have transient responses of NREMS amount, depth and consolidation. Females, in contrast, demonstrated significantly decreased NREMS, which was surprising, given the significantly higher amount of total sleep females exhibited compared to males during baseline conditions. Perhaps since female degus are more reactive to stress, an increase in cortisol following deprivation may have caused a significant decrease in NREMS during recovery. In addition, the prolonged elevation of SWA in response to the night deprivation may have been a more efficient recovery mechanism that females utilize to compensate for the loss of sleep amount. Examination of the underlying neurobiological mechanisms may provide insight into this sex-specific recovery method.

Slow Wave Activity- Insight into Sleep Function?

While sleep research has demonstrated hormones directly influence amount and consolidation of sleep, all sleep parameters are not directly modulated by steroids.   SWA regulation functions independently of circulating hormones, as many studies have failed to establish sleep intensity increases in conjunction with other sleep parameters in the presence of endogenous or exogenous hormones (Shechter & Boivin, 2010 ; Driver et al., 1996). Instead, perhaps dissimilar rates of homeostatic buildup (as a function of sex, age, or morning-evening chronotype) and varying rates of exponential decay between sexes account for this sexually dimorphic pattern of recovery. Given that morning/evening chronotype influences decay rates of SWA (Mongrain et al.,

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2006), a factor such as sex may also play a prominent role in the disparities observed across recovery.

A more intriguing possibility for this disparity of SWA may be linked to its hypothesized function. A recently published theory (Tononi & Cirelli, 2003; Tononi & Cirelli, 2006) posits synaptic strength mediates SWA. In light of the synaptic homeostasis theory, sex differences in sleep intensity may be controlled by synaptic strength. Does this mean that females have stronger synaptic association? It may just be indicative of the higher baseline SWA level of females, yet lack any functional significance. However, the fact that females (humans and degus) recover more efficiently from sleep deprivation seems to suggest it does have functional relevance. Additionally, female degus had low levels of SWA during their light phase and may have had to sleep more to attempt to achieve homeostatic balance. The synaptic homeostasis hypothesis has not been explored between sexes, and may provide insight into sexually dimorphic mechanisms of sleep. In view of the fact that thalamic Ca^{2+} channels regulate SWA and are mediated by estrogen, this may be important for elucidating the direct and indirect role of hormones in sleep regulation.

Additionally, recent work has elucidated two distinct components of slow waveforms (Lee et al., 2004; Massimini et al., 2009). Thalamic gating mediates the classic slow delta waveform, but recent focus in the synaptic homeostasis field has moved to the "slow waves" within the frequency range of less than 1Hz, which are cortically generated. Most sleep research does not distinguish

between delta waves (0.5-4.5Hz) and traditional slow waves (< 1Hz), as studies have focused on delta power given its established role as an indicator of homeostatic pressure. Recent studies have examined these two waveforms separately and found that they are separately regulated (Steriade et al., 1993; Sanchez-Vives & McCormick, 2000; Lee et al., 2004). Delta waves appear to be directly associated with synaptic homeostasis, while the characteristic slow waves are more influenced by typical somnogens like adenosine and prostaglandins (Massimini et al., 2009).   My data cannot rule out the possibility that female degu sleep data is likely partially mediated by prostaglandins (and other somnogens), in addition to steroid hormones.

Sleep and Development

Previous human and animal data clearly showed SWA amplitude decreases with advancing age, peaking during puberty and declining rapidly in young adulthood and further deteriorating with aging (Feinberg et al., 1990; Manber & Armitage, 1999; Carrier et al., 2001; Koehl et al., 2006). The new emerging theory of synaptic homeostasis suggests a framework to understand these findings (Tononi & Cirelli, 2006) that the time course of synaptic plasticity follows the decline in slow wave activity robustly (Feinberg et al., 1990). My data support this, with male degus demonstrating a powerful decrease in SWA across the lifespan within our studies. Juvenile male degus (pre-puberty) have a strong classical SWA rhythmicity which declines with advancing age.   Previous work suggests SWA changes during the lifespan, which appears to follow cortical

synaptic density (Feinberg, 1989; Feinberg et al., 1990). During the sleep state, the brain becomes the ideal environment for synaptic scaling via long term depression (Chen, 2000; Tononi & Cirelli, 2006; Czarnecki et al., 2007), which may provide a functional purpose for this decline across development, as synaptic pruning decreases and becomes less important with increased maturation.

Additionally, hormone influence on the rhythmicity of sleep during puberty may play an important role in this SWA activity pattern. The thalamus is widely accepted as the site of generation of delta waves, sleep spindles and SWA characteristic of NREMS.   Sleep architecture and SWA during development may be altered by differential hormonal gating of thalamic calcium (Ca^{2+}) channels (Crunelli et al., 2006; Qiu et al., 2006) and thalamic Ca^{2+} channels modulate the generation of delta waves and sleep spindles. Additionally, these thalamic Ca^{2+} channels are definitely mediated by estrogen (Lee et al., 2004; Crunelli et al., 2006), but the role of androgens in the gating of T-type Ca^{2+} channels remains unclear and should be investigated further to elucidate its possible role in the decline of SWA.

Elegant work by Branchey and colleagues (1973) indicates that gonadadectomy during development permanently alters sleep in adulthood, but castration in adulthood has no significant impact on sleep in rats. These results suggest a critical window for circulating hormones to influence sleep and our six-

month-old EEG data support this hypothesis.   Circadian variation of NREMS and REMS occurs exclusively in this age group.

Surprisingly, I discovered juvenile degus do not appear to have more REMS than adults, as most sleep studies have demonstrated REMS dominates sleep architecture during infancy and childhood and decreases with age (Frank & Heller, 1997). As previously discussed in the sleep and development chapter, a myriad of factors may have influenced these findings. The most likely factor is the relatively low REMS sleep need within this species. This appears to be species specific, as most other nocturnal and diurnal animals have higher amounts of REMS. An interesting possibility for the lack of REMS may be associated with the VLPO of degus. Specifically, the extended area of this sleep regulatory nucleus may provide clues to the apparent lack of REMS homeostasis. My preliminary neuroanatomy data highlighted the low levels of fos-expressing neurons within the extended VLPO during "sleep" collection times during the dark phase. A small amount of "sleep active" galanergic neurons in the shell of the VLPO may result in low levels of REMS in this animal model, as galanin expressing neurons project to the TMN, which is widely accepted as the pathway by which the VLPO influences sleep amount and timing (Saper et al., 2005).

Moreover, recent work has implicated orexin in REMS regulation (Kantor et al., 2009), which may be another pathway that is responsible for the circadian control of REMS within degus. Orexin neurons have not been examined in and

may represent an intriguing area of further exploration of REMS homeostasis within this unique animal model. Lastly, NREMS and REMS have been shown to serve different functions and this may also account for the apparent low REMS need. For example, NREMS has been more associated with declarative memory (Marshall et al., 2006), while REMS has been linked to spatial and procedural memory (Tambini et al., 2010 ; Stickgold, 2005). This may indicate low REMS could be an evolutionary adaptation of these animals, signaling degus may have less need for spatial memory (hence low REMS) or synaptic plasticity may be more dependent on synaptic homeostasis induced by high SWA levels. Additionally, it may simply be the result of different sleep circuitry within this species.

Sleep Circuitry

Previous work has investigated the differences in retinal projections and sleep circuitry within a variety of species. During my preliminary neuroanatomical experiments (data not shown), I discovered that degus lack a direct retinal projection to the VLPO (by utilizing both monosynaptic and multisynaptic tract tracers). This is a stark contrast to findings from nocturnal rodents, which reveal profuse retinal fibers innervate the VLPO (Lu et al., 1999). Light promotes sleep in night active animals, so the lack of retinal projections to the VLPO in the diurnal degu was expected. Additionally, other studies show differences in the retinal projections to other areas involved in sleep-wake regulation (Fite & Janusonis, 2001). Both nocturnal and diurnal animals have retinal projections to

the SCN along the retino-hypothalamic tract (RHT) (Moore, 1993). Yet while both nocturnal and diurnal animals have similar retinal innervations to the circadian pacemaker, the subsequent rhythms are 180 degrees out of phase with one another, suggesting a downstream mechanism for determining preferred patterns (Kas &Edgar, 1999; Smale et al., 2003). My preliminary evidence of diurnal/nocturnal disparities in projections to sleep-wake regulation areas reinforced this hypothesis and may provide clues to these possible downstream mechanisms. Schwartz and Nunez (2005) found sparse retinal fibers in the grass rat, suggesting there may be important differences within various diurnal animal models. The grass rat shares more circadian functional commonalities with the nocturnal rat than other diurnal mammals, but these neuroanatomical differences may play a vital role in their diurnality. Additionally, retinal projections and sleep/wake area efferents may influence the development of diurnality in the degus and provide insight for further sleep studies with degus.

The classical view of the sleep research field posited the SCN mediated the timing of sleep (Edgar et al., 1993; Easton et al., 2004) and VLPO mediated the sleep amount, particularly NREMS (Saper et al., 2005). An emerging view challenges this widely held theory. Research findings have revealed the VLPO may mediate not only amount of NREMS, but also NREMS timing and REMS amount (Chou et al., 2002; Gaus et al., 2002; Lu et al., 2002). The core VLPO has been implicated in NREMS regulation and the extended VLPO has been involved in REMS regulation. Previous neuroanatomical work has highlighted sleep-activated galanin cells within this area (Gaus et al., 2002), as galanin

positive sleep active VLPO neurons project to the TMN, which is prominently involved in arousal. My preliminary data highlighted the low levels of fos-expressing neurons within the extended VLPO during "sleep" collection times during the night. A small amount of "sleep active" galanergic neurons in the shell of the VLPO may result in low levels of REMS in this animal model. Furthermore, estrogen and testosterone have been shown to reduce REMS amount, which may account for the low levels within the adult degus. Additional work should investigate the level and functionality of hormone receptors within the extended VLPO, as these factors may mediate not only the low levels within the species, but the high disparity between sexes. These brain areas merit further exploration to identify their specific role in regulation of sleep-wake rhythms, as well as to illuminate how they potentially differ across nocturnal and diurnal chronotypes. These areas serve as ideal target sites for investigating possible downstream mediators of diurnality/nocturnality.

Future Directions

There has been a resurgence of interest in sex differences within sleep research. Increased interest within this area facilitates exploration of the impact of hormones on sleep regulation and further enables investigation of possible underlying neurobiological mechanisms. Many sleep disruptions have demonstrated different incidence levels and divergent presentation of symptoms across sex, which has fostered additional research aimed toward investigating the sex differences in sleep. My sleep studies examining the circadian and

homeostatic drive between sexes are the only studies to date that have addressed these topics in a diurnal rodent. No research has investigated the impact of hormones on sleep regulation in a diurnal rodent between sexes. These data may have identified the particularly powerful influence of female hormones on sleep in this species. Future studies should examine these sleep parameters in gonadectomized female and male degus with hormone replacement to ascertain the specific role of hormones in sleep regulation for this species. This will also enable investigation of the influence of hormones under steady state conditions, eliminating pulsatile hormone effects that may be circadian or cycle dependent.

Future work should also address the role neuroanatomy plays in sleep regulation of diurnal and nocturnal animals. Most brain areas involved in sleep/ wake regulation explored in neuroanatomical studies have illuminated varying innervations based on niche. The VLPO, a hypothalamic nuclei key for sleep promotion, has shown a stronger direct retinal projection in the rat, but not the degu. Conversely, the dorsal raphe nuclei (DRN) has demonstrated a stronger retinal input within the diurnal degu, compared to the nocturnal rat (Fite et al., 1999; Fite et al., 2001). Future research should examine the efferents of these key nuclei, which may provide insight into neuroanatomically distinct pathways that serve as the foundation for diurnal/nocturnal differences in sleep.

Additionally, there is an increasingly prevalent view that posits an integrated homeostatic/circadian regulation of sleep instead of the classic

"opponent process" view that has been maintained previously (Franken & Dijk, 2009). This may provide important insight into the development of diurnal or nocturnal niche and its role in sleep regulation. Furthermore, not only could possible differences in direct retinal projections to hypothalamic and other sleep promoting nuclei influence the sleep processes separately, but may have a synergistic function that further impacts sleep regulation. Mathematical modeling (of homeostatic exponential decay rates and circadian components of sleep) based on the current degu sleep data may further elucidate these sex differences and highlight different compensatory mechanisms for recovery of lost sleep. In addition, I can examine how these mechanisms may change over the lifespan and between sexes, as well as elucidate how the sleep homeostatic and circadian processes may interact. This will help illuminate whether these processes are distinct or more integrated in this diurnal animal model. My forthcoming mathematical modeling work examining the homeostatic and circadian sleep drive and their interaction may be particularly insightful in light of this emerging integrated view of sleep processes. Future studies should also aim to address these topics by concurrently performing neuroanatomical, molecular and cellular experiments.

Implications

My aforementioned work has highlighted that degus can serve as a diurnal sleep model for humans. The sex differences displayed in the sleep of these animals under baseline and disrupted conditions was particularly interesting and

complex. The complicated picture that emerged has highlighted why there has been a lack of sex differences research. There are many factors that modulate sleep-wake patterns outside of biological sex, and it is a daunting task to attempt to unravel the complex networks and mechanisms underlying sleep regulation. Additionally, these factors have been little explored in diurnal animals (besides humans) because of the expense and difficulty involved in establishing and maintaining a colony of day-active species. Previous research examining sleep in diurnal mammals has utilized chipmunks, squirrels, grass rats and monkeys (Dijk & Daan, 1989; Edgar et al., 1993; Schwartz & Smale, 2005). Yet the difficulty breeding diurnal rodents (the majority were wild caught) and the lengthy time investment and high financial cost of studying diurnal primates (squirrel monkeys) have hindered more sleep studies from being performed in diurnal animals.

Octodon degus breed easily in captivity and are relatively inexpensive to maintain. In addition, previous research has demonstrated the many commonalities between humans and degus, such as time course of development, female reproductive cycle and regulation of circadian rhythms to name a few. My work has highlighted the similarities and differences in the sleep regulation of humans and degus across development and between sexes. Of particular interest is the possibility that this diurnal rodent provides an ideal animal model for investigating sex differences that could be acutely relevant for humans. Although this area has been largely neglected, the rise in sleep research utilizing and focusing on females has been heartening. The field of

sleep research, and neuroscience more broadly, is recognizing the need for inclusion of females in experiments, especially with mounting evidence that women are more likely to complain of sleep problems and have higher incidence levels of insomnia and mood disorder-related sleep disturbance.

Furthermore, the recent discoveries linking SWA to synaptic strength may provide meaningful insight into the sexually dimorphic nature of sleep intensity. Innovative work has utilized transcranial magnetic stimulation (TMS) within the thalamus to generate an increase in slow waves, NREMS amount, and by extension, SWA (Massimini et al., 2007). Yet, due to the novelty of this research, any potential benefits of artificially induced enhancement of NREMS have not been examined or quantified. This may have important implications for learning and memory, cognitive functioning, and emotion/mood regulation in addition to sleep. This animal model could serve as an intermediary to examine possibly efficacious treatments in a diurnal mammal, which may have translational research value for functional sleep modeling and eventual treatment. This could be particularly relevant because NREMS reduction has been directly linked with obesity, diabetes and cardiovascular issues and therefore represents a major public health issue.

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