THE ROLE OF RAPID EYE MOVEMENT AND SLOW WAVE SLEEP FOR THE CONSOLIDATION OF MEMORY IN RATS

by

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Abstract

The functions of sleep remain enigmatic. One of the dominant, yet more contentious hypotheses is that sleep is involved in memory consolidation. A large body of evidence supports the role of rapid eye movement (REM) sleep in memory consolidation, especially in rodents. In humans, the role of REM sleep in memory consolidation has also been investigated, however it is unclear if it supports only one type of memory, or consolidation for several memory systems. Recent evidence suggests that non-REM is also involved in memory consolidation. The role of theta activity during REM and sleep spindles during non-REM may provide electrophysiological signatures reflecting memory consolidation processes. The studies presented here attempt to further investigate the electrophysiological characteristics of the learning-dependent changes in REM and slow wave sleep (SWS) in rats. A 2-stage model of memory consolidation is outlined here, and both steps of the model were investigated. Consistent with previous studies, REM increases were observed following avoidance training. During this period, theta power during REM sleep was increased compared to non-learning rats. Increased sleep spindle density during SWS was observed following REM increases. When REM sleep was suppressed by infusing the GABA$_B$ agonist baclofen into the pedunculopontine nucleus, avoidance performance acquisition was impaired. Baseline sleep spindles predicted whether rats were able to learn to make avoidance responses. Results suggest that both REM and SWS may be sequentially involved in memory consolidation processes. Discrete periods (windows) exist for REM and SWS when memory consolidation processes appear to take place. Theta activity during REM sleep from 17-20 h on the first post-training day and sleep spindles during SWS from 21-24 h on the first post-training day are increased in learning rats and are related to memory performance.
Co-Authorship


Chapter 3 is in publication and can be cited as Fogel, S. M., Smith, C. T., & Beninger, R. J. (in press). Increased GABAergic activity in the region of the pedunculopontine and deep mesencephalic reticular nuclei reduces REM sleep and impairs learning in rats. Behavioral Neuroscience.

Chapter 4 has been submitted for publication and can be cited as: Fogel, S. M., Smith, C. T., & Beninger, R. J. (submitted). Too much of a good thing? Elevated baseline sleep spindles predict poor avoidance performance in rats.
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Statement of Originality

I hereby certify that all of the work described within this thesis is the original work of the author. Any published (or unpublished) ideas and/or techniques from the work of others are fully acknowledged in accordance with the standard referencing practices.

(Stuart M. Fogel)

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List of Abbreviations

AASM: American Academy of Sleep Medicine
ANOVA: analysis of variance
BL: baseline
DpMe: deep mesencephalic reticular nucleus
EEG: electroencephalogram
EMG: electromyogram
EOG: electrooculogram
FFT: fast fourier transform
fMRI: functional magnetic resonance imaging
GABA: gamma-aminobutyric acid
IQ: intelligence quotient
LDT: laterodorsal tegmentum
LG: learning group
LTP: long-term potentiation
NLG: non-learning group
NMDA: N-methyl-D-aspartic acid
p-wave: pontine component of the ponto-geniculo-occipital wave
PET: positron emission tomography
PGO: ponto-geniculo-occipital
PPN: pedunculopontine nucleus
RBD: rapid eye movement behaviour disorder
REM: rapid eye movement
RSW: rapid eye movement sleep window
SD: standard deviation
SWS: slow wave sleep
TS: transition sleep
Chapter 1
General Introduction

Preface

The overall goal of this dissertation was first, to generate a testable model from characterizing the electrophysiological learning-dependent changes in sleep in the rat, and second, to test this model using pharmacological manipulations, targeting brain structures involved in the generation and maintenance of sleep states. Chapter 1 provides a description of the characteristics of sleep. This is followed by a brief overview of the relevant memory literature and a review of the literature relevant to the sleep and memory hypothesis both in humans and animals. Chapter 2 is the first of three manuscripts where learning-dependent changes in sleep architecture and spectral power across the sleep cycle are characterized. A 2-stage model of sleep and memory consolidation is proposed involving both rapid eye movement (REM) and non-REM sleep. The first step in the proposed 2-stage model was tested with the use of pharmacological manipulations of REM sleep (chapter 3). Chapter 4 investigated what characteristics of sleep at baseline predict subsequent performance improvements. Finally, chapter 5 summarizes the main findings of chapters 2 through 4, discussing the implications, limitations, shortcomings and future directions of this line of research.

Sleep States

In studying the functions of sleep, it is important to recognize that sleep is not a passive absence of wakefulness and loss of consciousness, but rather, consists of a physiologically complex, highly regulated series of stages. There are characteristic behavioural, cognitive and physiological features associated with each stage, particularly as recorded in the electroencephalogram (EEG).
Virtually all animal life exhibits some sort of diurnal rest-activity cycle. All mammals exhibit both REM and non-REM sleep. Birds exhibit less REM and non-REM sleep than most mammals, reptiles exhibit only signs of non-REM sleep and amphibians and fish do not experience sleep *per se*, but do have a diurnal rest-activity cycle. In all mammalian species, non-REM and REM occur in an alternating cycle, with non-REM sleep invariably preceding REM sleep. REM sleep is present in many birds, thought to have evolved from reptiles that exhibit only non-REM sleep (for review see Campbell and Tobler, 1984). Thus, REM sleep seems to have evolved separately in mammals and birds, perhaps to serve similar functions. In mammals, several factors appear to account for varying duration and patterns of sleep, including predator-prey status, habitat type, body size and life-span. Vulnerability to predators is increased during sleep, and thus it is not surprising that animals that can be clearly categorized as prey (e.g., deer: 3 hours sleep per day) sleep less than animals that can be categorized as predators (e.g., lion: 14 hours sleep per day). Conversely, other theories predict that sleep serves a protective function from predation, as some species of mammals (e.g., rodents) have adapted to sleep more than 12 hours per day. One resolution to this issue is to take into consideration the type of habitat. Many species have adapted to sleep in surroundings that offer protection from predators (e.g., burrows; tree tops), and accordingly sleep more hours per day than those who sleep in less protective surroundings (e.g., open plains; ocean). For example, the eastern chipmunk sleeps for approximately 16 hours per day, whereas the pilot whale sleeps for only 5 hours per day.

In humans, the various stages of sleep cycle over the course of a night (Figure 1). During the first half of the night slow wave sleep (SWS) predominates, whereas REM sleep predominates in the latter half of the night. Stage 2 sleep is distributed relatively evenly throughout the night. A night of sleep can be broken down into roughly 90-minute cycles comprised of a non-REM component that terminates with a period of REM sleep. Rats in
laboratory settings, on the other hand, are polyphasic sleepers. They sleep in small bouts (from several seconds up to about 10 minutes) with many transitions between wake, SWS and REM which are dispersed throughout the 24-hour cycle. Rats tend to have at least one bout of consolidated wakefulness near the start of the dark cycle when they are most active (Borbély, 1980).

Figure 1. A typical hypnogram of human sleep architecture (right) and corresponding EEG which characterize each sleep stage (left). Slow wave sleep (Stages 3 and 4) predominate the first half of the night, whereas Stage 2 and REM predominate the second half of the night.

Each sleep state can be characterized by its unique electrophysiological features, normally measured by the EEG recorded from the scalp to measure the changes in brain activity across sleep states, electromyogram (EMG) to measure changes in muscle tension associated with changes in sleep state and electrooculogram (EOG) primarily to identify the characteristic rapid eye movements of REM sleep (Figure 2). REM sleep is characterized by the presence of low-voltage, rapid, desynchronized oscillations in the cortex that resemble those of the waking state. It is for this reason that the term paradoxical sleep was coined to describe this state in cats (Jouvet, Michel, and Courjon, 1959). Unlike waking, the cortical EEG of REM sleep is accompanied by paralysis of the body musculature, and particular electrophysiological patterns (theta rhythm; 5–8 Hz), originating in the hippocampus (a structure that is closely tied to acquisition and storage of
Figure 2. Typical human polysomnographic recordings including EEG (upper trace), EOG (middle trace) and EMG (lower trace) in each sleep-wake stage.
new information). One of the most salient signs of REM sleep is the presence of rapid eye movements first described in humans by Aserinsky and Kleitman, (1953) and by Dement who recognized REM sleep as the state which is now commonly associated with a high probability of dreaming activity (Dement and Kleitman, 1957). There are several stages of non-REM sleep, usually designated stages 1 – 4 in humans (although more recently, distinguishing between stages 3 and 4 is no longer recommended in clinical settings; AASM; Iber, 2007). The deepest levels of non-REM sleep (stages 3 and 4) are characterized by high-voltage, slow oscillations called \textit{delta waves} (1–4 Hz), which are absent or infrequent in other stages, and therefore stages 3 and 4 are often collectively called SWS. In rodents (Figure 3), often, no distinction between Stage 2 sleep and SWS is made, thus non-REM sleep is typically called SWS, although some identify light and deep non-REM sleep as well as the transition from SWS to REM sleep as separate states in the rodent (Gottesmann, 1996). In humans, stage 2 sleep, which occupies approximately half of the sleep night, is characterized by the presence of other unique electrophysiological markers called sleep spindles (Gibbs and Gibbs, 1950) and k-complexes (Roth, Shaw and Green, 1956). Sleep spindles are phasic events in the EEG that oscillate in the 11–16 Hz range, have a fusiform shape, tapered at either end, with a maximal amplitude in the middle. In young adults, spindle bursts typically last from about 0.25 to 1.5 seconds, have a mean frequency of 14 Hz and occur on average every 12 seconds and have an amplitude of about 21 µV (Principe and Smith, 1982; Silverstein and Levy, 1975).
Figure 3. Typical rat polysomnographic recordings including EEG (upper trace) and EMG (lower trace) in each sleep-wake stage (wake, slow wave sleep (SWS) and rapid eye movement (REM) sleep.

Memory Systems

A brief overview of the memory systems to be discussed follows. This is intended only to provide sufficient background to discuss the sleep and memory literature and to provide adequate definitions for the terminology used here.

Ribot (1882) was the first to observe that the degree of memory loss following a brain injury depended on the age of the memory, with more recent memories affected more so than remote memories. Penfield, Milner and Scoville (1957, 1958) first observed amnesia for facts and events, but intact skill learning in individuals with medial temporal lobe damage. Particular patterns of memory deficits are observed with damage to particular brain structures (for review see Gabrielli, 1998). These observations were further refined by Squire and colleagues (Squire, 1992), leading to the compartmentalization of memory into distinct systems broadly described as declarative and procedural memory systems. Declarative memory refers to memory for knowing...
“what” whereas procedural memory refers to memory for knowing “how”. Declarative memory includes memory for facts and events, which are usually learned explicitly (i.e., consciously learned and recollected) and are commonly referred to as semantic or episodic memory. A commonly used task to assess declarative learning is the paired associates task whereby individuals are asked to memorize word pairs presented in sequence, and then are subsequently presented the first word of each word pair and asked to recollect the second word from memory. Procedural, or non-declarative memory includes memory for motor skills, habits, procedures, priming and conditioning (including stimulus-response, and incentive learning) which is usually learned implicitly (i.e., independent of conscious learning and retrieval).

In the sleep and memory literature, a useful distinction between the various subtypes of procedural memory tasks proposed by Smith (1995, 2001) has been between cognitive procedural memory and simple procedural memory tasks. Cognitive procedural learning involves improved performance according to a rule or strategy that is learned implicitly from performing the task and can be either verbal (e.g., Wff ‘n’ Proof Task) or non-verbal in nature (e.g., Mirror Trace Task). Simple procedural learning is not cognitively complex and involves improved performance with practice usually on a sensorimotor task (e.g., Pursuit Rotor Task). The distinction between cognitive procedural learning and simple procedural learning is mostly based on task characteristics. Traditionally, memory systems are classified according to the pattern of cognitive deficits observed in brain-injured patients and in neurodegenerative disease. There is little clinical neuropsychological evidence to distinguish between cognitive and simple procedural memory and many of these tasks have been considered to be a part of the same neuropsychological classification system (Gabrielli, 1998). However, the dissociable learning-related changes in sleep suggest that these procedural memory subtypes are subserved by different memory structures.
Newly acquired memories are initially in a labile state, vulnerable to interference and amnesic influences. With time, memories become transformed into a more stable form through the process of consolidation. Lesion studies have demonstrated that circumscribed lesions to the medial temporal lobe (including the hippocampus) result in temporally graded retrograde amnesia (Squire, Slater, and Chace, 1975, for review see Squire and Alvarez, 1995). The hippocampus is thought to have a time-limited role in the storage of memories. There are at least two main perspectives describing the role of the hippocampus and extra-hippocampal structures (such as the neocortex) in the process of consolidation: 1) consolidation theory and 2) multiple trace theory. Consolidation theory stipulates that the hippocampus temporarily stores information and is involved in transforming this information into a more permanent form in the neocortex. Initially, sensory information is processed in cortical areas that subserve these functions, and the hippocampus maintains the pattern of activation that makes up a particular memory trace. Over time, hippocampal-neocortical reactivation strengthens the cortico-cortico connections, and the influence of the hippocampus diminishes. The result is a memory trace that is more independent of the hippocampus and more integrated into existing cortical networks. An alternative theory is based on findings that following hippocampal lesions, memory for semantic information showed temporally graded retroactive amnesia whereas memory for episodic information had a flat gradient for recent and remote memories (Nadel and Moscovitch, 1997). Thus, one of the tenets of multiple trace theory is that the hippocampus is responsible for providing the spatial and temporal context in which the memory trace was initially learned, and that retrieval of contextually-dependent memory depends on the reactivation (and transformation see Moscovitch et al., 2005; Winocur et al., 2007) of the hippocampal-neocortical trace. Remote semantic memories that are largely context-independent can be retrieved independently of the hippocampus, and thus preserved following hippocampal damage.
Sleep States and Memory Systems

What mechanisms are involved in the process of consolidating newly learned information into a more stable form of long-term storage? Sleep has been identified as one of the biological states that contribute to efficient memory consolidation. A compelling body of research exists from both animal (for reviews see Smith, 1985; 1996; Giuditta, 2003) and human studies (for reviews see Diekelmann, 2009; Peigneux and Smith, in press; Stickgold and Walker 2007; Maquet et al., 2003) establishing a link between REM sleep and memory consolidation. Despite this, an ongoing (and sometimes heated) debate continues about the many functions of sleep (Maquet, 2001; Siegel, 2001; Stickgold and Walker, 2005a; Stickgold and Walker, 2005b; Vertes and Siegel 2005a; Vertes and Siegel, 2005b; Walker, 2005). More recently, the focus has turned increasingly towards non-REM sleep which has also been implicated in the consolidation of newly learned material (Gais and Born, 2004; Nader and Smith, 2003; Smith and MacNeill, 1994).

There are at least three main approaches used to study the relationship between sleep and memory consolidation: 1) total or selective sleep deprivation and restriction following learning, 2) sleep recording (typically EEG studies, but more recently accompanied by brain imaging) following learning, compared to baseline recordings or performance control groups, and 3) sleep enhancement following learning.

The sleep deprivation paradigm is useful to identify whether sleep is required at all for a particular type of memory consolidation to occur. One of the first investigations of sleep and memory was by Jenkins and Dallenbach (1929) who found that sleep improved retention of non-sense words, as compared to across an equivalent period of wakefulness. Individuals were instructed to study a list of non-sense words, followed by an interval filled with either wake or
sleep. Retention for the number of non-sense words was then tested after the wake/sleep interval to determine if sleep improved recall (or conversely, at the time it was believed that sleep prevented forgetting as a result of interference during wake). One of the flaws with this type of paradigm is that training and testing are conducted at different circadian phases. Despite this drawback, similar paradigms are still used to identify what types of learning require sleep for optimal consolidation.

Similar approaches take advantage of the fact that SWS and REM sleep predominate the first and second half of the night, respectively. Thus, you can have retention intervals filled with either wake or sleep in the first half of the night (mostly Stage 2 and SWS) and compare it to retention intervals filled with either wake or sleep in the second half of the night (mostly Stage 2 and REM). There are several drawbacks to this approach including differences in circadian time of testing, levels of sleep pressure during testing and levels of sleep inertia following sleep. Other sleep deprivation techniques attempt to reconcile these issues by training individuals before an interval of sleep, followed by sleep deprivation whereby either REM, non-REM, Stage 2 or SWS are selectively disrupted. Testing is then conducted the next day.

Daytime napping studies can also be used to investigate the differential benefit of sleep for learning. Similar to the early/late paradigm, different length naps will contain different types of sleep. A very short duration nap (i.e., 20 minutes) will likely contain only stage 1 and 2 sleep. A somewhat longer nap may contain in addition to Stages 1 and 2, stages 3 and 4. A nap of over 60 minutes in length would likely also contain REM sleep. All of the sleep deprivation techniques may also not completely control for interference during time spent awake, and the added stress and fatigue associated with sleep deprivation. The major strength of deprivation studies is also their major drawback: while these studies are instrumental in identifying which type of sleep is necessary for a given type of learning, they do not provide any information about the
characteristics of sleep involved in consolidation processes. This is one of the reasons that sleep recording techniques are used to corroborate evidence from sleep deprivation studies (and vice-versa).

Sleep recording studies typically use within subjects designs whereby post-learning sleep is compared to baseline sleep. Sleep can also be compared to non-learning (or performance) controls. The recording paradigm is especially useful as it is not subject to the drawbacks of the deprivation paradigm, and it permits the investigation of the characteristics of sleep that may be potential markers or mechanisms of sleep-dependent memory consolidation. Another advantage to the recording paradigm is that a variety of recording techniques can be used such as electrophysiological (scalp recorded, epidural, implanted) and functional neuroimaging (positron emission tomography (PET), functional magnetic resonance imaging (fMRI); for review see Maquet et al., 2003), or most recently, coregistered EEG and fMRI (the advantage being both high temporal and spatial resolution afforded by each of the respective techniques; Schabus et al., 2007). If post-training sleep is involved in memory consolidation, then changes to sleep architecture (e.g., increases in a particular stage of sleep), the electrophysiological features of sleep (e.g., sleep spindles during non-REM sleep, rapid eye movements during REM sleep or increased power using spectral analysis techniques) can be identified. Using functional neuroimaging, reactivation of brain structures that support memory processes can also be identified.

A third class of techniques involves modifications to post-training sleep. The goal of this paradigm is typically to enhance sleep, or to provide a cue during post-training sleep to subsequently enhance memory. One of the first studies to use this approach involved training individuals on an artificial grammar task while a clicking noise was present in the background (Smith and Weeden, 1990). Auditory clicking cues delivered to coincide with rapid eye
movements during REM sleep enhanced subsequent performance on the task. When auditory clicks were presented during REM, but not coinciding with eye movements, no enhancement was observed. Not only does this type of study provide evidence that a particular type of sleep may be involved in memory consolidation, but it also provides causal evidence suggesting that phasic events such as rapid eye movements may be markers of consolidation processes. Other studies have used olfactory cues (Rasch et al., 2007), neurofeedback techniques (Hoedlmoser et al., 2008), transcranial magnetic stimulation (Bergmann et al., 2008; Marshall et al., 2006) and electrical stimulation of the scalp (Marshall et al., 2005) to successfully enhance sleep-dependent memory consolidation.

The State-Dependent Hypothesis

A large body of research exists to support the role of REM sleep in memory consolidation (for review see Gais and Born, 2004 and Smith, 2001; Smith, Aubrey and Peters, 2007 for an alternative perspective). Historically, SWS was considered to largely subserve physical restoration as this stage of sleep predominates during periods of physical growth and is one of the only times when growth hormone is released. Stage 2 sleep until recently has been regarded as relatively unimportant as compared to SWS and REM sleep, reflected by the labels non-REM sleep, “filler sleep” and “non-restorative sleep”. The state-dependent hypothesis posits that different types of sleep contribute to the consolidation of different types of memory. Much of the evidence supporting this hypothesis comes from the human literature, which is reviewed here.

Cognitive procedural learning and sleep. Buchegger and Meier-Koll (1988) conducted one of the first studies to look specifically at motor skills learning and changes in human sleep. In this study, they used a trampoline training program as the novel motor skills learning task. There was an increase in the minutes of REM sleep, and changes in the non-REM/REM sleep cycle
after eight separate training sessions compared to baseline sleep. A second study investigated whether changes in REM sleep were learning- or performance-related using a physical activity control group (Buchegger et al., 1991). The total duration of REM sleep increased following trampolining compared to baseline and physical activity controls, supporting the original hypothesis that REM sleep is important for cognitive procedural memory, but not physical activity alone.

The Wff’ n Proof logic task involves coding skills according to an artificial grammar and is considered a cognitive procedural task (Smith, 1995). Selective REM sleep deprivation impairs performance on this task (Smith and Lapp, 1986; Smith, 1993), which suggests that REM sleep may be involved in the consolidation of cognitive procedural memory. It was hypothesized that rapid eye movements might play a role in memory consolidation. In one of the few studies of its kind, Smith and Weeden (1990) found that cues coinciding with rapid eye movements themselves during REM sleep enhanced subsequent task performance (see above). Results indicated that REM sleep is involved in procedural learning requiring complex logic skills and that the rapid eye movements themselves are involved in the consolidation process. Recently, Rasch et al. (2007) used a similar approach, utilizing olfactory cues during either SWS or REM sleep for both a declarative and a procedural memory task. It was found that when the olfactory cue was presented during SWS, declarative memory was enhanced, however cue presentation during REM sleep did not enhance subsequent memory performance. These findings suggest that SWS is involved in declarative memory consolidation and perhaps not REM sleep. However, the procedural task used in this experiment was a task thought to be Stage 2 sleep dependent (Nader and Smith, 2003), and olfactory cues were not presented during Stage 2 sleep, nor was a REM–dependent cognitive procedural task used. Thus, the findings from cueing studies appear to have identified a role for REM sleep and SWS in cognitive procedural and declarative memory consolidation, respectively.
It also remains to be tested whether cueing during Stage 2 sleep may enhance simple procedural learning.

Plihal and Born (1997) found that a retention interval with a high proportion of REM sleep late in the night was associated with improved cognitive procedural memory performance on the mirror tracing task, compared to those who had early sleep or waking controls. Tweed et al. (1999) selectively deprived individuals of REM or disrupted Stage 2 sleep and found that REM sleep deprivation impaired subsequent performance on the mirror-tracing task relative to groups exposed to Stage 2 sleep disruption or to normally rested controls. These results support the hypothesis that REM sleep is involved in the consolidation of cognitively complex procedural memory skills, and are consistent with the results of Plihal and Born (1997).

A series of studies using neuroimaging techniques have also been used to investigate learning-dependent changes in sleep (Maquet et al., 2003). Recently, Peigneux et al. (2003), conducted a study to investigate changes in regional cerebral activation using PET following the acquisition of a motor sequential artificial grammar task. The task used in this study (Cleeremans and McClelland, 1991) required participants to quickly and accurately press one of six keys corresponding to the position of one of six stimuli displayed on a computer screen. The sequence of the stimuli was determined by an artificial grammar not known to the participants. The next stimulus in the sequence could be implicitly learned and predicted from the previous stimulus. A performance control group was tested using a completely random sequence of stimuli. This way, it could be determined whether cerebral reactivation during REM sleep was the result of reprocessing the artificial grammar or simply visuomotor skill acquisition. It was found that the brain structures (i.e., cuneus and striatum) active during the practice session were reactivated during REM sleep in the group that performed the sequential grammar task compared to the random task or controls that did not perform the task. From these results, it was concluded that
reactivation during REM sleep may represent reprocessing of newly learned skills.

Walker et al. (2002) have shown that when sleep in individuals intervenes between testing and re-testing for a sequential finger tapping task, enhanced performance is observed compared to participants exposed to equal intervals filled only with waking. Performance was shown to be related to the amount of Stage 2 sleep in the last quarter of the night. Conversely, Fischer et al. (2002), using a similar task, found that REM sleep was associated with performance improvements. The question remains, is REM sleep or Stage 2 sleep necessary for this type of learning? Clarification may be found in the task characteristics used in these experiments. Fischer et al. (2002) used a bi-manual version of the finger tapping task, which may have increased the complexity of the task. This added complexity may have required REM sleep for efficient memory consolidation. Kuriyama, Stickgold and Walker (2004) designed a study specifically to address this issue. They found that when the task complexity was increased (i.e., bimanual vs. unimanual and long vs. short sequences) sleep benefit increased. It remains to be tested whether different types of sleep were differentially associated with the task improvements for low complexity vs. high complexity motor tasks. Smith, Nixon and Nader (2004) found that postlearning performance on cognitive procedural tasks was correlated with REM density increases. Furthermore, they found that this relationship was strongest in individuals with high intelligence quotient (IQ) scores. These findings indicate that learning-dependent changes in sleep observed in high skill individuals may be larger than low skill individuals.

Currently, there is a relative consensus for the involvement of REM sleep in cognitive procedural memory. The results summarized above have been interpreted to mean that REM sleep is involved in memory consolidation for learning that requires the acquisition of a new strategy or rule. However, it may be that more generally, REM sleep may be involved in the
consolidation of learning that is novel. Thus, prior experience with the type of material would better dissociate between whether the learning was REM-dependent or non-REM-dependent.

**Simple procedural learning and sleep.** One of the earliest studies to directly investigate the relationship between Stage 2 sleep and simple procedural memory was by Smith and MacNeill (1994). They deprived four independent groups of either REM sleep, non-REM sleep, sleep deprivation confined to the last half of the night only or total sleep deprivation. It was found that following total sleep deprivation or sleep deprivation in the last half of the night, performance on the pursuit rotor was significantly impaired compared to fully rested controls and REM-deprived subjects. Since the last half of the night is made up almost entirely of either Stage 2 sleep or REM sleep and since pursuit rotor performance was unaffected by selective REM deprivation, it was deduced that Stage 2 sleep is necessary for normal simple procedural memory consolidation.

To investigate the relationship between Stage 2 sleep and simple procedural memory Smith and Fazekas (1997) used a similar paradigm and deprived participants of either total (Stage 2 and REM) sleep for the entire last half of the night or only REM sleep in the last half of the night. Following sleep deprivation in the last half of the night, performance on the pursuit rotor was impaired compared to fully rested controls or the REM deprived group. In addition, the selectively REM deprived group had some Stage 2 sleep interruption as a result of REM sleep awakenings. This group performed at an intermediate level on the pursuit rotor based on the Stage 2 sleep disruption. Similarly, Tweed et al. (1999) investigated the effects of Stage 2 sleep and REM sleep deprivation on memory. Three sleep groups were exposed to selective REM sleep deprivation, Stage 2 sleep interruption or no sleep deprivation for the entire night. It was found that Stage 2 sleep interruption impaired performance on the simple tracing task compared to either the REM deprived group or normally rested controls, indicating that Stage 2 sleep
disruption may impair simple procedural memory.

In addition to the pursuit rotor and simple tracing task, Karni et al. (1995) observed performance improvements on the finger tapping task after an intervening 24-hour period between training and retesting. Walker et al. (2002, 2003) designed an experiment to investigate whether the slow improvement on the finger tapping task observed over a 24-hour period was sleep dependent. Participants were trained on the finger tapping task in the morning. When re-tested 12 hours later, only marginal improvements in performance were observed. However, when re-tested following an intervening night of sleep, a large gain in performance was observed. To ensure that the lack of improvement during the interval filled with wakefulness was not due to interference from performing other motor activity throughout the day, a comparison group was required to wear mittens to restrict the amount of motor activity during this period. This group showed the same small performance improvement following the wake interval and the same large increase in performance following a night of sleep. An additional group was trained and re-tested at different intervals to determine if sleep *per se* or the passage of time was responsible for the increase in performance on the finger tapping task and to control for circadian effects on task performance. A large increase in performance was observed when participants were trained in the evening and re-tested in the morning. However, when trained in the morning and re-tested in the evening with an intervening period of wakefulness, no increase in performance was observed. Thus, the passage of time alone does not seem to contribute to performance improvements, rather simple procedural learning is sleep-dependent. Importantly, it was also found that the duration of Stage 2 sleep was correlated with overnight improvement and this relationship was especially strong during the second half of the night.

Long-term potentiation (LTP) is one of the potential mechanisms involved in the consolidation of memory (Ivanco and Racine, 2000; Trepel and Racine, 1998) and is thought to
involve Hebbian-type principles (Hebb, 1949). LTP of synaptic pathways occurs in networks of neurons by strengthening synaptic efficacy both pre- and post-synaptically. Neural correlates of LTP include increased dendritic growth, decreased synaptic distance, increased neurotransmitter reuptake, and more efficient axonal conduction by increased mylenation (for review, Gustafsson and Wigstrom, 1988). The findings reported thus far provide evidence that Stage 2 sleep is necessary for the consolidation of simple procedural learning. One of the electrophysiological markers of Stage 2 sleep is the sleep spindle. Sleep spindles have been suggested to be a likely mechanism for the consolidation of new learning (Steriade and Amzica, 1998; Steriade, 1999; Destexhe and Sejnowski, 2001; Rosanova and Ulrich, 2005). However, none of the previously mentioned studies have systematically investigated the effect of simple procedural learning on sleep spindles using a recording paradigm. Sleep spindles have been considered ideal for the induction of LTP in the cortex for several reasons. During the sleep spindle, there is a large influx of calcium ions into cortical cells. The influx of calcium ions is the mechanism for LTP of cortical cells (Ghosh and Greenberg, 1995). This influx of calcium may serve to prime the synapses for permanent changes (Destexhe and Sejnowski, 2001). It has been proposed that this state may also be involved in the expression of genes related to synaptic plasticity (Li et al., 1998).

Recently, there has been a great deal of interest in the function of the sleep spindle for memory consolidation. Fogel and Smith (2001, 2006) investigated the effects of simple procedural learning on Stage 2 sleep and sleep spindles (using a variety of simple procedural learning tasks including the pursuit rotor, simple tracing task, ball-and-cup task, and the board game “Operation”). It was found that the number of sleep spindles, spindle density (spindles per minute), sigma power (12 to 14 Hz) and duration of Stage 2 sleep increased as a result of simple procedural learning compared to non-learning controls. In addition, there was no change in the
density of rapid eye movements during REM sleep, which indicated that phasic markers of REM sleep were not affected by simple procedural learning. We concluded that Stage 2 sleep is important for the efficient consolidation of simple procedural memory. Sleep spindle activity is a marker for this type of memory consolidation and possibly a mechanism for cortical LTP. The results of this study were later replicated (Fogel, Smith and Cote, 2007) using the pursuit rotor task alone and increases were found in spindle density as well as the average duration of the sleep spindle. Further, sigma power increased over frontal regions in the second half of the night during Stage 2 sleep and persisted into SWS. Peters, Smith and Smith (2007), using the same task, grouped subjects according to skill level and found that in the high skill group, spindle density increased following pursuit rotor learning. Additionally, sleep spindles were correlated with re-test performance in the high skill group, whereas REM density was correlated with re-test performance in the low skill group. The relationship between procedural learning and sleep spindles has also been demonstrated in a daytime nap. The number of sleep spindles and sigma power correlated with motor performance following the nap, for habitual nappers but not for those who do not habitually nap during the daytime (Milner, Fogel and Cote, 2007). The relationship between sigma power and motor skills improvement was strongest over central (C3, Cz, C4) and parietal (P3, Pz, P4) regions. Recently, the relationship between performance on the finger tapping task and sleep spindles was found to be strongest in the right hemisphere during a daytime nap (Nishida and Walker, 2007). Together these findings suggest that learning dependent changes in spindle activity during a daytime nap are similar to those observed over an entire night of sleep (Mednick et al., 2002; 2003).

In summary, Stage 2 sleep appears to be important for the consolidation of simple procedural learning, and more specifically, sleep spindles appear to be involved in the consolidation process. Simple procedural learning does not appear to be affected by REM sleep
deprivation, and is associated with Stage 2 sleep in the latter part of the night. Other studies using declarative tasks have also found a role for Stage 2 sleep in declarative memory consolidation which will be reviewed in the next section. It may be the case that the cognitive versus simple dissociation may not be the essential distinction, rather the novelty or level of previous experience with similar types of learning may be more important. Thus, sleep stage 2 sleep and sleep spindles may serve to consolidate refined skill expertise.

**Declarative learning and sleep.** To investigate the relationship between sleep and declarative memory, Barrett and Ekstrand (1972) used retention intervals filled primarily with either Stage 4, REM sleep or no sleep in three independent groups. The results indicated that performance on a paired-associates task was better when the retention interval was filled with sleep as opposed to no sleep. In addition, it was found that sleep in the first half of the night was more beneficial than sleep in the last half of the night for paired-associates learning. These findings suggest that SWS sleep may be beneficial to declarative learning rather than REM sleep. Grosvenor and Lack (1984) argued that these studies (Barrett and Ekstrand, 1972; Ekstrand et al., 1971; Fowler, Sullivan and Ekstrand 1973; Yarouch et al., 1971) confounded the type of sleep that followed the learning sessions with the type of sleep preceding learning. When prior sleep was controlled for, it was found that early sleep was not as important for memory as Barrett and Ekstrand initially thought. Using the same early versus late sleep testing paradigm, Plihal and Born (1997) investigated the effects of retention intervals filled with either early or late sleep or early or late wakefulness on declarative learning performance. Plihal and Born (1997) confirmed the work done previously by Ekstrand et al. (1971). Early sleep with proportionately more SWS was associated with improved declarative memory performance compared to retention intervals filled with wakefulness early in the night, late in the night, or with sleep late in the night that had proportionately more REM sleep. Despite the drawbacks of using the early versus late sleep
testing paradigm (discussed in the previous section), together with converging evidence from other experimental designs, these findings suggest that SWS is involved in the consolidation of new declarative learning.

Declarative memory is initially hippocampal dependent (Winocur, 1990). Animal studies have shown that hippocampal dependent memory can be acquired quickly (Racine, Milgram and Hafner, 1983) and has a faster rate of decay than in the neocortex (Trepel and Racine, 1998). However, more lasting storage of declarative memory requires slower hippocampal-cortical transfer of information (Nadel and Moscovitch, 1997; Rosenbaum, Winocur and Moscovitch, 2001; Zola-Morgan and Squire, 1990). Theta is suggested as the frequency that the hippocampus utilizes to communicate with the other structures (Klimesch, 1999) and is involved in the induction of LTP (Larson, Wong and Lynch, 1986). It has been suggested (Born and Gais, 2003) that this process is facilitated by sleep and would occur slowly over several nights. It has been suggested that theta activity is a marker for hippocampal-neocortical dialogue (Siapas, Lubenov and Wilson, 2005; Buzsáki, 1996). Fogel, Smith and Cote (2007) found that theta power increased during REM sleep, but not during non-REM sleep following paired-associates learning compared to non-learning controls and to cognitive procedural and simple procedural groups, suggesting that theta may be involved in declarative memory consolidation processes during REM sleep. These findings appear to be at odds with each other, and it is still unclear whether declarative learning is SWS-dependent or REM sleep dependent.

There is also evidence to suggest that Stage 2 sleep is important for declarative memory consolidation. The recording paradigm has also been used to test whether declarative learning is related to increases in Stage 2 sleep spindles. In this study, Gais et al. (2002) found that spindle density in the first half of the night increased following declarative learning on a paired-associates task. Spindle density (spindles/minute) was positively correlated with recall of paired-associates
before and after sleep. These results are inconsistent with previous studies that did not identify a role for Stage 2 sleep in declarative learning (for review see Smith, 2001). It is important to note that the paired associates task used in this study did not present word-pairs in sequence, rather they were presented in blocks of eight word-pairs. Thus, it is unclear exactly what type of mnemonic strategy was used by the participants. Another group (Schabus et al., 2004) used the paired associates task, presenting word pairs in sequence and instructing the participants to use a particular mnemonic strategy utilizing visual imagery to relate the words to one another. No change in sleep spindles was observed when all subjects were grouped together. However, they noticed that some of the subjects increased their spindles following learning, whereas others did not. When separated into two groups based on learning-related changes in spindles, it was found that the “spindle enhancer” group had improved performance following sleep, whereas the “non-spindle enhancer” group did not. The difference in sleep spindles from baseline to the test night was correlated with improved paired associates recall. This result has since been confirmed by Clemens et al. (2005; 2006). Since these variables are correlated, the opposite approach could also have been taken. Subjects could have been split into groups according to task improvement, or baseline performance level as opposed to changes in spindles, thus producing a low and a high skill group. It would be expected that the high skill group would have had a learning-dependent change in sleep spindles, whereas the low skill group would have had little or no change in sleep spindles. Thus, similar to procedural learning, initial skill level may be important for assessing the type of learning-dependent changes in sleep resulting from declarative learning. Schabus et al. (2008) conducted a study to examine this hypothesis and found that individuals who improved on the memory task had an increase in sleep spindles following declarative learning, whereas non-improvers had no change in spindles. This result occurred independent of assessed general memory skills (Advanced Progressive Matrices test).
In summary, there is only moderate consensus among researchers regarding the role of sleep for declarative learning. This presents a major stumbling-block for the sleep state-dependent hypothesis of memory consolidation as sleep does not have a clearly defined role for one of the two major types of memory (i.e., declarative vs. procedural), and there is little neuropsychological evidence from brain injured patients to distinguish between simple and cognitive procedural learning. However, there is considerable evidence to suggest that SWS, REM and Stage 2 sleep may all have a role in declarative memory consolidation. It is possible that all types of sleep contribute to declarative learning. Declarative memories are initially hippocampal dependent. Declarative memories become dependent on neocortical structures and less dependent on the hippocampus over time as they become increasingly integrated with other memories (Nadel and Moscovitch, 1997; Rosenbaum, Winocur and Moscovitch, 2001; Zola-Morgan and Squire, 1990). Thus, declarative memory consolidation is a slow process that involves the medial temporal lobe, neocortical and subcortical structures over time. In order for consolidation processes to occur, and if it is sleep related, it would be hypothesized that several or all types of sleep may be necessary for declarative memory consolidation to occur.

**Novel vs. familiar learning and sleep.** An alternative explanation for the discrepancies in the literature could be that the level of expertise with a given task is an important factor for the nature of the learning-dependent changes in sleep. Smith, Aubrey and Peters (2004) proposed that complex tasks require REM sleep, whereas the refinement of simple and already learned or existing skills would require Stage 2 sleep. It was hypothesized that the initial skill level of the individual would determine what stage of sleep would be necessary to consolidate new learning. For example, low skill individuals would learn new skills, thus post-learning changes in REM sleep would be hypothesized. On the other hand, high skill individuals would refine existing skills, thus post-learning changes in Stage 2 sleep would be expected. While this approach may
help to resolve some of the discrepancies for procedural tasks considered complex or simple, it is not clear whether this approach will resolve the inconsistencies observed with declarative learning. Studies where subjects are assigned to groups based on fixed factors such as intelligence, general memory abilities, or according to learning-related changes in sleep spindles, indicate that sleep spindles may perform some function for memory consolidation but only for individuals who are able to proficiently learn the task, irrespective of general memory abilities. Conversely, it is possible, that memory performance improves only in those who have increased sleep spindle activity after initial task acquisition. It remains unclear whether initial skill level explains whether declarative learning-dependent changes in sleep will favor SWS, REM or Stage 2 sleep.

**Animal Studies of Sleep and Memory**

The investigation of sleep and memory consolidation is currently in a renaissance period. Renewed interest in this area began with the discovery of REM sleep in 1953 (Aserinsky and Kleitman, 1953). This discovery led to investigations of the functional significance of REM sleep, and to the development of one of the dominant hypotheses: that REM sleep is involved in memory consolidation. Some of the earliest investigations were carried out in animals by Bloch (for review, 1970), Fishbein et al. (for review, 1977), Pearlman et al. (for review, 1979) and Smith et al. (for review, 1985). Collectively, they produced a compelling body of converging evidence using both sleep deprivation and sleep recording techniques that post-learning REM sleep is involved with memory consolidation. Many of these studies only recorded or deprived sleep for the first few hours after training, and despite this limitation, found much support for the involvement of REM sleep in memory consolidation. Smith et al. (1974) and Fishbein et al. (1974) were the first to demonstrate that more pronounced increases in REM sleep occur at longer delays following training (Figure 4). REM deprivation during these periods of enhanced
REM sleep was shown to impair memory performance, and these periods were termed REM sleep windows (RSW, for review see Hennevin et al., 1995; Smith, 1995; 1996; 2003).

Figure 4. A hypnogram displaying the frequency of sleep-wake stages (wake, slow wave sleep (SWS), and rapid eye movement (REM) sleep) scored in 30-second epochs across a 24-hour period. The hypnogram is an example of post-training sleep architecture for a rat that was able to successfully learn (over 60% correct avoidances) in the two-way shuttle avoidance task. The time period encircled from about 14 to 20 hours indicates the RSW for this task and training regime.

**REM deprivation studies.** The most commonly used approach to study the role of sleep in memory consolidation was to selectively deprive REM sleep. The rationale for this approach was that if REM sleep was involved in memory consolidation, then REM deprivation should disrupt the consolidation process, and impair subsequent memory performance. In the 2-way shuttle avoidance task, for example, rats were trained for 100 trials in one day (Smith, Young and Young, 1980), 50 trials/day over 2 days (Smith and Lapp, 1986), or 20 trials/day over 5 days (Smith, Young and Young, 1980). Each group was REM sleep deprived for 4-hours during different 4-hour periods following the last training trial and memory performance was tested to identify the timing of the RSW. When rats were trained in one session, a RSW was found at 1-4 hours post training. After two sessions, RSWs were found at 9-12 and 53-56 hours after training. After 5 sessions, RSWs were found at 9-12 and 17-20 hours. When REM deprivation occurred
outside these periods, subsequent memory performance was not significantly affected. Furthermore, if REM sleep was only allowed during the RSW, but prevented for the remainder of the post-training period, no significant impairment in performance was observed compared to rested controls (Smith and Butler, 1982; Smith, Young and Young, 1980). In general, the more condensed the training, the shorter the latency of the RSW. The duration of the RSW appears to be unaffected by the training regime, and for the shuttle avoidance task, has a minimum duration of 3 hours (Smith and Bustos, 1992). Thus RSWs are discrete periods, about 4 hours in duration when sleep deprivation during these periods impairs subsequent performance, and the timing of the RSW varies according to the intensity of the training regime.

Using the Morris water maze, a similar pattern has been observed (Smith and Rose, 1992; Smith, Conway and Rose, 1993) whereby REM deprivation impaired performance at earlier delays (1-4 hours) in the more condensed training condition (12 trials in 1 day) as compared to REM deprivation at later delays (5-8 hours) in the more protacted training condition (4 trials/day for 4 days). RSWs have also been found for the radial arm maze (Legault, Smith and Beninger, 2004, 2006; Smith and Conway, 1998). Thus, the timing of the RSW varies both as a function of the type of task and the intensity of the training regime for the given task. Increased REM sleep also predominates during the RSW, which is reviewed in the following section.

**Sleep recording studies.** The other major approach used to study the role of sleep in memory consolidation is to record sleep before and after training using polysomnographic recordings. The rationale for this approach is that if post-learning sleep is involved in memory consolidation, learning-related changes in sleep should be observed compared to baseline sleep and non-learning controls exposed to the same training conditions. Increases in REM sleep have been observed following shuttle avoidance training (Lucero, 1970; Hennevin and Leconte, 1971; for review see Smith, 1985). Increased REM sleep does not appear to be related to some non-
specific effect of the training such as fatigue, fear or excitement as increased REM sleep was only observed in animals that were able to successfully learn the task and not in animals exposed to the same training regime that were unable to learn the task. The timing of REM sleep increase depends on the intensity of the training regime. When trained for 50 trials/day for 2 days on the shuttle avoidance task, increased REM sleep was observed from 9-12 hours after training. On the second post-training day, no change in REM sleep over baseline was observed. However, when sleep was recorded for several days, increased REM sleep was maximal 5 days after training and lasted 7 days (Smith and Lapp, 1986).

In some cases, increased REM was observed in the first few hours following training (Bloch, 1970; Lucero, 1970; Portell-Cortes et al., 1989). When training on the shuttle avoidance task is protracted, REM increases are observed at later delays (Fishbein, Kastaniotis and Chattman, 1974; Smith and Lapp, 1986) and improvement varies between animals. When aligned according to the maximum increase in performance, increased REM sleep is observed the day before performance improves (Bloch, Hennevin and Leconte, 1979; Smith, Young and Young, 1980). Using an appetitive task (Smith and Wong, 1991), rats were trained to perform bar-presses to get a food reward using a fixed ratio reward schedule. All rats learned the task at the same rate. However, only some of the rats had increased REM sleep following training. When trained on a more complex appetitive task, requiring that bar presses are made in a particular sequence, only the rats who demonstrated increased REM sleep following the simpler task were able to learn the more complex task. Thus, animals showing REM sleep increases on the simpler tasks could be identified as having more learning potential than those that did not.

From the 1980’s to mid-1990’s interest in sleep and memory diminished, largely due to inconsistencies in the literature, heavy criticism regarding stress related to the memory tasks and sleep deprivation techniques. Many of these issues remain unresolved and heated debates
continue on the topic (Maquet, 2001; Siegel, 2001; Stickgold and Walker, 2005a; Stickgold and Walker, 2005b; Vertes and Siegel 2005a; Vertes and Siegel, 2005b; Walker, 2005). One study in particular by Wilson and McNaughton (1994) helped to reignite interest in the sleep and memory hypothesis by demonstrating that pairs of hippocampal cells which were coactive during spatial navigation, and have a receptive field for a particular spatial location (“place cells”), were reactivated during SWS sleep (although the initial discovery of this phenomena can be attributed to Pavlides and Winson, 1989). In a series of studies, Wilson and McNaughton (1993; 1994) recorded the activity of place cells while rats navigated a circular track. They observed that during subsequent SWS sleep, the same pattern of activation observed during exploration was present in the same temporal order (albeit on a compressed timescale). Activation of these cells was correlated with the occurrence of sharp-wave ripples (Kudrimoti et al., 1999), which coincide with sleep spindles in the neocortex (Siapas and Wilson, 1998). Sleep spindles have been regarded as a candidate mechanism for synaptic consolidation (Destexhe and Sejnowski, 2001) and several recent studies have found post-learning increases in ripple activity (Mölle et al., 2009) and spindles in humans following procedural (Fogel and Smith, 2006; Fogel, Smith and Cote, 2007) and declarative learning (Gais et al., 2002; Schabus et al., 2004). In rats, learning-dependent increases in sleep spindles have been observed following novel object presentation (Schiffelholz and Aldenhoff, 2002) and reward learning (Eschenko et al., 2006).

Another sleep EEG marker of memory consolidation is the p-wave. Datta et al. (for review see Datta and Patterson, 2003) have studied the potential functional significance of the p-wave, the pontine component of the ponto-geniculo-occipital (POG) wave observed in the cat (Brookes and Bizzi, 1963; Jeannerod, Mouret and Jouvet, 1965). In the rat, p-waves last 75-100 ms in duration and have an amplitude of 100-150 µV. They occur in bursts of 3-5 waves/burst, and at a rate of 30-60 waves per minutes in REM sleep (Datta et al., 1998). The p-wave
predominates during REM sleep and the transition to REM sleep. Datta et al. provide compelling evidence that the p-wave is responsible for the reactivation of cortical memory traces during sleep. The induction of cortical LTP requires spaced and repeated patterned stimulation (Larson et al., 1986; Trepel and Racine, 1998). One of the few endogenous rhythms during REM sleep that provide similar stimulation is the p-wave, originating from pontine regions of the midbrain. Retrograde labeling techniques identified output structures of p-wave generating cholinergic cells, most notably, the occipital cortex, entorhinal cortex, piriform cortex, hippocampus, amygdala and other brainstem structures involved in the generation of REM sleep (Steriade and McCarley, 1990). Behavioural and electrophysiological evidence from a recent study (Datta, 2000) investigated changes in p-wave generation following shock avoidance training in either avoidable or unavoidable shock conditions. Rats in the avoidable condition demonstrated learning within 30 trials with an average of over 90% correct avoidances. Compared to controls, the learning group had a significant increase of about 20% in p-waves during REM sleep, and over a 180% increase over controls during the transition to REM sleep. The degree of improvement was highly, and positively correlated with the increase in p-waves. These results suggest that p-waves may serve as a marker, or potential mechanism for memory consolidation not only during REM sleep, but also especially so during the transition from SWS to REM sleep. A similar mechanism may exist in the human brain.

The sequential hypothesis. One of the earliest and most influential hypotheses that included both REM and non-REM sleep in memory consolidation was proposed by Giuditta (1977; 1985) and was recently elaborated (Giuditta et al., 2003). Briefly, the sequential hypothesis proposes that sequences of sleep stages (for example; SWS → transition sleep → REM) change in frequency depending on learning performance. The main underlying premise is that one type of sleep alone is not sufficient to allow consolidation processes to occur, but rather,
certain sequences of sleep transitions are important to allow both the suppression of redundant or competing information and the enhancement of behaviourally relevant information. The sequential hypothesis examines each transition from one state to the next in exhaustive combinations of transitions, and trains of these transitions. In this way, the role of particular transitions for memory consolidation can be identified.

Giuditta’s hypothesis suggests that a 2-stage process may be involved in memory consolidation. One of the defining characteristics of REM sleep is the theta rhythm, which is also implicated in hippocampal-dependent memory processes (Vertes, 2005). Thus, learning-related increases in REM sleep produce increases in theta activity. As memory consolidation progresses, the role of the hippocampus diminishes as the memory trace becomes increasingly strengthened in neocortical areas (Nadel and Moscovitch, 1997). Sleep spindles have been suggested as a likely mechanism for neocortical consolidation (Fogel and Smith, 2006; Fogel, Smith and Cote, 2007; Gais et al., 2002; Mölle et al., 2009; Schabus et al., 2004). Thus, it is hypothesized that increased REM sleep (and theta activity during REM sleep) would be followed by increases in sleep spindle activity during SWS. REM sleep may be involved in consolidating the newly learned and behaviourally relevant aspects of the task. This is putatively followed by a period of refinement during SWS whereby sleep spindles may be involved in further consolidation processes. Increases in theta power may represent hippocampal-neocortical communication (Buzsáki, 1996), whereas increased sleep spindle activity may represent subsequent thalamocortical activity to induce LTP in the neocortex (Steriade and Amzica, 1998; Steriade, 1999; Destexhe and Sejnowski, 2001; Rosanova and Ulrich, 2005). These two processes together would result in the transfer of newly learned information from the medial temporal lobe to the neocortex. Thus the proposed 2-stage hypothesis assumes that first, REM sleep is involved in transferring newly
learned information to cortical networks (indicated by increased theta rhythms), which are subsequently refined during SWS (indicated by increased spindles).

The research reviewed in this chapter suggest that more than one sleep state may play a role in memory consolidation, and given the historical focus on REM sleep, particularly in nonhuman animals, the learning-dependent changes in both REM and non-REM remain to be investigated in greater detail. The main goal of the following studies is to 1) further characterize the learning-dependent changes in both REM and non-REM sleep across the entire 24-hour cycle in hopes of generating a testable model of sleep-dependent memory consolidation, 2) identify markers of sleep-dependent memory consolidation from epidural EEG recordings, and 3) through the use of pharmacological manipulations of these markers, test hypotheses generated from this model of sleep and memory.
Chapter 2
Evidence for 2-stage Models of Sleep and Memory: Learning-dependent
Changes in Spindles and Theta in Rats

The role of sleep in memory consolidation has been a contentious topic in the neurosciences (Walker, 2005). Particular stages of sleep and their unique electrophysiological characteristics have been implicated in neural plasticity. However, the time-course of the learning-dependent changes in sleep have not been well characterized, nor are the relative contributions of the various sleep stages to memory consolidation processes well understood.

Paradoxical or rapid eye movement (REM) sleep plays a significant role in the formation of new memories (Smith, 2003). During REM sleep following learning, memories may be consolidated by long-term potentiation (LTP)–like processes (Bramham, Maho and Laroche, 1994). Without REM sleep, performance improvements are smaller in humans (Smith, 1996) and animals (Smith, 1985; Smith, 2003; Hennevin et al., 1995). Changes in the number of REMs and in theta activity, which characterize REM sleep, have been observed in humans following learning (Fogel, Smith and Cote, 2007; Smith and Lapp, 1991; Smith Nixon and Nader, 2004). Thus, REM sleep contributes to memory processing.

Following learning, increases in REM sleep have been observed (Smith, 1985; Smith, 2003). In rats, these increases do not occur homogeneously across an entire 24-hour period (or more) following training. Rather, REM increases occur in discrete periods, which have been termed REM sleep windows (RSWs). RSWs are periods of sleep characterized by increased number of REM sleep episodes, thereby increasing the total duration of REM sleep following learning (Smith, 1985; Smith, 2003). The timing of the RSW systematically varied as a function

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of animal strain (Smith, Lowe and Smith, 1977), type of memory task (Smith and Rose, 1997; Smith, Young and Young, 1980) and the training regime (Smith, Young and Young, 1980). The RSW has typically been observed to be limited in duration to about 4 hours. REM sleep deprivation for a period of 4 h during but not outside of the RSW impaired memory (Smith, 2003). The electrophysiological learning-related changes during the RSW have yet to be investigated, and were the major focus of this investigation.

In humans, REM sleep deprivation and reduced rapid eye movements (REMs) resulted in memory impairment (Smith, 1993; Smith, 1996; Smith and Smith, 2003). Memory consolidation may be enhanced during learning-dependent increases in REM sleep. During REM sleep, ensembles of hippocampal neurons were reactivated in the same pattern as during initial learning (Wilson and McNaughton, 1994). P-waves (the pontine component of the ponto-geniculo-occipital circuit that triggers REMs) and REMs in the rat were correlated with theta power during REM sleep (Karashima et al., 2004). Thus, learning-dependent increases in REMs and theta may reflect brain activation involved in memory consolidation. It is not known whether learning-dependent changes in theta power accompany increases in REM sleep during the RSW following learning.

More recently, the role of Stage 2 sleep in memory consolidation has been investigated. In humans, Stage 2 sleep makes up about 60% of a night’s sleep and is characterized by sleep spindles, k-complexes and less than 30% delta wave activity. The electroencephalogram (EEG) appears more synchronized and generally slower than waking. Muscle activity is lower than waking, but higher than the muscle atonia observed in REM sleep. In humans, Stage 2 sleep deprivation impairs simple procedural memory performance on tasks such as the pursuit rotor (Smith and MacNeill, 1994), and increases in Stage 2 sleep have been observed following periods of simple procedural learning (Fogel an Smith, 2006; Fogel, Smith and Cote, 2007). Sleep
spindles characterize Stage 2 sleep, and until recently, their function has remained elusive. Following periods of simple procedural learning (Fogel an Smith, 2006; Fogel, Smith and Cote, 2007) or verbal learning (Clemens, Fabó and Halász, 2005; Gais et al., 2002) increases in sleep spindles have been observed in humans. In animals, increases in sleep spindles in a 60-min period immediately following associative learning (odor-reward pairing task) have also been observed (Eschenko et al., 2006).

The goal of the present study was to investigate the learning-dependent changes in EEG spectral power to better characterize the changes in brain activity that may reflect memory consolidation processes across the entire 24 h post-training day. We examined the hypothesis that increases in theta power and sleep spindles would occur in the RSW following learning. This investigation may provide additional information about the processes involved in sleep-dependent memory consolidation and provide experimental evidence to support a 2-stage model for sleep-dependent memory consolidation proposed in humans (Smith, Aubrey and Peters, 2004) and animals (Buzsaki, 1996; Sirota and Buzsaki, 2005). Thus, according to this model both REM and non-REM sleep may be involved in memory consolidation.

**Methods**

**Methods summary**

Twenty male Sprague-Dawley rats weighing 250-300 g were implanted with four EEG and two electromyogram (EMG) electrodes. Animals were housed with ad libitum food and water and kept on a 12 h light-dark cycle. After 14 days of recovery, 3 days of acclimatization, and 24 hours of baseline (BL) recording, animals were trained on the two-way avoidance task for 100 trials (50 trials/day) and re-tested for 25 trials on day 3. EEG was recorded for 22 h after training on both training day 1 and 2. EEG was stage scored as REM, SWS or wake, analyzed using a fast
Fourier transform (FFT) algorithm, and sleep spindles were automatically counted. Rats in the learning group (LG) (n=8) avoided footshock on 60% of the test trails. The remaining rats (n=12) were assigned to the non-learning group (NLG).

**Animals and housing**

Twenty male Sprague-Dawley rats (Charles River, Saint-Constant, Quebec, Canada) weighing 250-300 g at the time of surgery were housed individually in opaque plastic cages (20 x 30 x 18 cm high) with food and water available ad libitum. Lights were on from 1100-2300 h, and off from 2300-1100 h. The experimental protocol was approved by the Trent University ethics review board.

**Surgery**

Anaesthesia was maintained with the use of isoflurane gas (0.5 L/min breathable oxygen with 1.5-3.0 % isoflurane/O2 maintained) delivered via a stereotaxic-mounted nosepiece. A nosepiece and ear bars restrained the head once Bregma and Lambda were levelled. Four EEG and two EMG electrodes were implanted. Each EEG electrode consisted of a stainless steel screw implanted into the skull attached to a 32 gauge (0.20 mm diameter), 2 cm long Teflon-coated stranded stainless steel wire. The two anterior screws were placed at stereotaxic coordinates 2 mm anterior and +/- 2.5 mm laterally from Bregma, overlying the motor cortex. The two posterior screws were placed at stereotaxic coordinates 3.5 mm posterior and +/- 3.0 mm laterally from Bregma. Posterior placement was overlying the hippocampus to better visualize theta. Two 32-gauge, 3 cm long bare stranded stainless steel wires were implanted into the neck muscles bilaterally. Each wire was crimped into a gold connector socket and routed to a six-channel connector affixed to the skull with dental acrylic. Each rat was administered an intramuscular injection of antibiotic and given liquid acetaminophen orally for post-operative pain relief. All
rats were allowed 14 days to recover from the surgical procedure and to become acclimatized to the cables attached to the skull connector.

**Sleep recording and analysis**

Sleep was recorded after the recovery period and after three consecutive days in the recording chamber with the skull connector attached to the recording cable. The first sleep recording session lasted 24 h and served as a baseline recording. During the recording sessions, the electrodes were connected from the skull connector on the rat to a commutator (Plastics One Inc.) that was then connected to a digital Sandman (Putritan Bennett Inc.) Suzanne™ polygraphic amplifier. Bipolar EEG recordings were made by referencing one of the two posterior sites to the anterior sites, chosen according to recording quality. The remaining posterior site served as an isolated ground electrode. EEG and EMG recordings were taken at 120 samples/second with a low frequency cut off at 0.18 Hz and a high frequency cut off at 42 Hz. A 60 Hz notch filter was also used. Sleep recording took place for 23-24 h starting between 1000 h and 1100 h. The recording apparatus consisted of a clear Plexiglas recording chamber (25 x25 x 50 cm high). Food, water and bedding were made available throughout the recording sessions.

For sleep scoring and analysis EEG was subsequently filtered using a 0.5 Hz high-pass filter and a 30 Hz low-pass filter. EMG was filtered using a 1 Hz high-pass filter and a 50 Hz low-pass filter. Sleep records were manually scored in 30 s epochs as wake, SWS or REM sleep when at least 50% of the epoch met the following criteria: wake - EEG appeared desynchronized, high frequency, low amplitude accompanied by elevated EMG activity; SWS - the appearance of sleep spindle activity, low frequency, high amplitude EEG, accompanied by lower EMG activity; REM sleep - the EEG was characterized by synchronized theta activity (6-10Hz), accompanied by lower EMG activity and the disappearance of sleep spindle and slow wave activity. Sleep
Spindles are high amplitude (>0.25uV) phasic waxing and waning (“fusiform”) events in the EEG from 12-16 Hz, typically lasting from 0.5 s up to 3 s. The transition between SWS and REM sleep, which is characterized by usually brief (<15 s) high amplitude bursts of sigma and theta activity were included with REM sleep scores. Transition sleep was not scored separately due to its short duration and was included with REM as this is a period marked by increased P-wave activity (a physiological characteristic of REM sleep) related to avoidance learning observed by Datta (2000). Sleep stage scoring was compared to stage scoring conducted in 10-s epochs to evaluate the reliability of scoring in 30-s epochs. One-hundred and sixty-two 30-s epochs were selected from baseline recordings from six different rats. Of the 162 epochs, there were 54 of each wake, SWS and REM (54x3=162). More specifically, four consecutive 30-s epochs (120 s) of data were pseudo-randomly sampled so that each of the 3 sleep stages/wake states were represented (e.g., Wake, SWS, REM). The 30-s epoch was rescored instead as three 10-s epochs. Rescoring was done by an experimenter who was blind to the original scoring of the 30-s epochs. Based on the 162 cases, the 30-s and 10-s scoring methods were in very close (92.0%) agreement (Kappa = .880, p < 1x10^-56).

The frequency content of the EEG was analyzed using FFT power spectral analysis. Only movement artifact-free EEG was included in the analysis. Movement artifact was identified using an automatic computer algorithm which identified periods of EEG in two separate passes for slow activity (1-6 Hz) and fast activity (18-60Hz) that was high amplitude and at least 2 s in duration. To accomplish this, the average amplitude of 3600 s of “background” EEG was calculated. When a window of at least 2 s of EEG within each 3600 s of EEG exceeded 4 standard deviations beyond the average amplitude, the 2 s window was labelled as artifact and subsequently merged with adjacent artifact events if closer than .10 s apart. The FFT analysis was done in 2 s overlapping windows (75% overlap), averaged into 30 s epochs so that each power value was
represented in the time scale used for sleep stage scoring. Four frequency bins were used including delta (1–5 Hz), theta (6–10 Hz), sigma (11–16 Hz) and beta (17–20 Hz) identified by Corsi-Cabrera et al. (2001). The spectral power values were then log transformed and multiplied by 10 to obtain units in decibels (dB), then averaged across each sleep-wake state within each 4 h recording period. EEG power for each sleep stage, in each 4 h period in each frequency bin was expressed as the change from baseline. When the assumption of sphericity was violated for ANOVA analyses, the degrees of freedom were adjusted using a Huynh-Feldt correction factor.

Sleep spindles were counted during SWS for the entire recording period on the baseline, training day 1 and training day 2 using a computerized algorithm. Sleep spindles were identified if they occurred in the 12–16 Hz range, were greater than 100µV in amplitude, had a minimum duration of 0.15 s, maximum duration of 3 s and a minimum inter-spindle interval of .25 s. Spindles identified with an inter-spindle interval of less than .25 s were merged and counted as a single spindle. The difference from baseline spindle density (number of spindles/min) was calculated for each 4 h period on training days 1 and 2. Only sleep spindles detected during movement artefact-free EEG using the same algorithm described above were included in the spindle count.

**Behavioural procedures**

Behavioural training and testing began at 9:00AM and ended by 11:00AM on training day 1, 2 and the retest on day 3 using a two-way shuttle shock avoidance task in a dimly lit room. The shuttle apparatus had two identical compartments (50 x 25 x 20 cm high). Each compartment had a house light and a floor which could be electrified to deliver a 1.5 mA foot shock. Behavioural training was preceded by a 5-min acclimatization period during which the rat was free to move from one compartment to another. The training sessions consisted of 100 trials
administered over 2 days (50 trials/day). The 50 trials on each day were separated into blocks of 25 trials by a 10-min break. Each trial began by illuminating the house light in the compartment occupied by the rat and sounding a tone for 10 s, followed by a 20 s (maximum) 1.5 mA scrambled 10-volt foot shock. The light, tone and foot shock terminated at the same time when either the rat travelled to the unlighted compartment, or the maximum trial duration expired. To avoid the foot shock, the rat had 10 s while the house light was illuminated to move to the unlighted compartment. Each trial was separated by a 10–20 s intertrial interval. Only data for the last 20 trails in each block were used in the analysis; the first 5 trials were treated as a warm-up period to allow rats to acclimate to the testing conditions and performance to stabilize.

**Statistical Analysis**

The sleep architecture, spindle analysis and FFT data were binned into the same 4-hour periods, aligned according to lights on (1100 h) and lights off (2300 h) and labeled periods 1-6 for each training day (1: 1100-1500 h, 2: 1500-1900 h, 3: 1900-2300 h, 4: 2300-0300 h, 5: 0300-0700 h, 6: 0700-1100 h). The last 1 to 2 hours (from 0900 to 1100 h) was when behavioural testing took place. The exact start time and duration of testing varied depending on the testing order of the cohort of rats, which was randomized. To account for the varying length of each time period, all sleep stages were expressed as the percent total recording time, and sleep spindles by the total duration in minutes of SWS sleep to yield a measure of spindle density (spindles/minute). The TRT did not vary between recording days.

Behavioural data were analyzed using mixed design 2 x 5 (group by block) ANOVA. A significant interaction was followed up by tests of simple effects of block for each group to test for significant changes in performance over blocks of trials. Sleep architecture, sleep spindle and FFT data were analyzed using a mixed design 2 x 6 (learning group by period) ANOVA.
significant interaction was followed up by testing simple effects to identify at what period significant group differences existed. In some cases, there was insufficient data in the first block of trials across animals, in which case a 2 x 5 ANOVA was used. When the assumption of homogeneity of variance was violated, the degrees of freedom were corrected using the Huynh-Feldt correction factor.

**Results**

**Behavioural Data**

Rats trained on a shuttle box avoidance task were easily distinguishable into learners and non-learners (Figure 5 and Figure 6). The learning group performed better than the non-learning group (Figure 6). A 2 x 3 (group x day) analysis of variance (ANOVA) revealed a significant interaction, the number of correct avoidances changing across the five testing blocks as a function of group (F(2, 36) = 4.64, p = 0.026). Tests of simple effects revealed that the learning group increased correct avoidances over days (F(2, 14) = 10.77, p < 0.007). There was no significant change in correct avoidances in the non-learning group.

**Learning-Dependent Changes in REM Sleep**

**Sleep architecture.** There was no significant difference in the percent duration of REM sleep between learners and non-learners on the baseline day (t(16) = 0.85, p = 0.41). In learners, an increase over baseline in the percent duration of REM sleep was observed from 13-16 h and 17-20 h following training on day 1 (Figure 7A). For training day 1, a 2 x 6 (group x period) mixed design ANOVA revealed a significant group by period interaction (F(5, 85) = 2.74, p = 0.02). Tests of simple effect of group at each period revealed that the learning group had significantly more REM sleep than the non-learning group from 13-16 h (t(17) = 2.24, p = 0.04)
Figure 5. Correct avoidances for each rat on the retest day in the learning (filled bars; n=8) and non-learning (open bars; n=12) groups. Dashed horizontal line indicates the 60% criterion. Data were rank-ordered by rat in descending order to illustrate the bi-modal nature of the distribution. There was no overlap between the groups.

Figure 6. Two-way shuttle performance in the learning (n=8) and non-learning (n=12) groups on the two training days and the re-test in the percentage of correct avoidances. Dashed horizontal line represents 60% correct performance criterion. Significant group differences (analysis of variance followed by tests of simple effects) indicated by * at p < 0.05.
and 17-20 h following training (t(17) = 3.66, p = 0.002). A similar ANOVA for training day 2 revealed no significant main effect of group or interaction. Results suggest that the RSW is a time when memory consolidation may take place beginning 13–20 h after training with the largest increase in REM taking place in the 17–20 h period.

The increased duration of REM sleep could have been due to either an increase in the number of REM episodes (defined as one or more consecutive 30 s epochs scored as REM) or by an increase in the duration of REM episodes (Table 1). T-tests revealed that the learning group had an increased number of REM episodes compared to the non-learning group 17-20 h following training (t(17) = 3.0, p < 0.01). There was no significant group difference for the average duration of REM episodes. Thus, results suggest that the increase in REM sleep duration observed in the learning group was attributable to an increase in the absolute number of REM episodes, but not an increase in the average duration of REM episodes.

Table 1. Means (M), standard deviations (SD) and t-test (t) results for number of REM episodes and the average duration of REM episodes in learning (n = 8) and non-learning rats (n = 11) on training day 1 in the 17-20 h period.

<table>
<thead>
<tr>
<th>REM episodes</th>
<th>Learning M</th>
<th>Learning SD</th>
<th>Non-Learning M</th>
<th>Non-Learning SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>14.0</td>
<td>4.1</td>
<td>6.5</td>
<td>6.1</td>
<td>3.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Average duration (min)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.2</td>
<td>0.8</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Power spectral analysis of the EEG. The largest changes in spectral power also occurred during the 17–20 h window on training day 1. From baseline to training day 1, the learning group showed large and significant increases in spectral power in the theta and beta frequency bands during REM sleep from 17-20 h and in the sigma and beta bands during REM sleep from 21–24 h (Figure 8A and B). There were no significant baseline group differences in theta power (t(15) = 0.26, p = 0.797). There was insufficient REM sleep to perform statistical analyses in the first 4 h.
Figure 7. Mean difference scores from baseline to training day 1 and 2 for the duration of rapid eye movement (REM) sleep (A), slow wave sleep (SWS) (B) and wakefulness (wake) (C) expressed as a percentage of total recording time within each 4-hour post-training recording period (1-6: 1-4 h, 5-8 h, 9-12 h, 13-16 h, 17-20 h, 21-24 h) in the learning (n=8) and non-learning (n=12) groups. Lights on were from periods 1-3, lights off from periods 4-6, indicated by the solid horizontal bar. Significant group differences (analysis of variance followed by tests of simple effects) indicated by * at p < 0.05.
period on training days 1 and 2. Thus for theta power on training day 1 a 2 x 5 (group x period) ANOVA found that during REM there was a significant group by period interaction ($F(4,60) = 2.83$, $p = 0.03$). Simple effects analysis revealed that the learning group had a larger increase in theta than the non-learning group from 17–20 h only ($t(15) = 2.43$, $p = 0.03$). There were no significant baseline differences in sigma power ($t(15) = 1.67$, $p = 0.116$) between learning and non-learning groups. For sigma power on training day 1, a significant group by period interaction was observed ($F(4,60) = 3.12$, $p = 0.05$), and tests of simple effects revealed that sigma power was significantly greater in the learning group from 21–24 h only ($t(15) = 2.90$, $p = 0.01$). There were no significant baseline group differences in beta power ($t(15) = 1.18$, $p = 0.258$). For beta power on training day 1, a significant group by period interaction was observed ($F(4,60) = 3.45$, $p = 0.03$), and groups were significantly different from 17–20 h and 21–24 h ($t(15) = 2.27$, $p = 0.038$; $t(15) = 2.44$, $p = 0.03$). Spectral power during REM sleep between learning and non-learning groups did not differ significantly on training day 2 in any frequency band (Figure 8A and B). There was no significant difference in delta power between groups on the baseline day ($t(15) = 1.32$, $p = 0.206$), or group by period interactions on either training day 1 ($F(4,60) = 2.06$, $p = 0.10$) or training day 2 ($F(4,60) = 0.07$, $p = 0.97$).

Learning-Dependent Changes in SWS and Wake.

Sleep Architecture. There was decreased SWS and increased wakefulness during the 21–24 h period in the learning group on the first post-training day, and a similar, but non-significant pattern on the second training day (Figure 7B and C). ANOVA revealed a significant group by period interaction on training day 1 ($F(5, 85) = 2.86$, $p = 0.03$). Simple effects ANOVA revealed that the learning group had significantly less SWS in the 21–24 h period than the non-learning group on day 1 ($t(17) = 3.16$, $p = 0.006$). Learners had significantly more SWS than non-learners on the baseline day ($t(16) = 2.82$, $p = 0.01$).
Figure 8. Mean change from baseline in electroencephalographic spectral power (dB) in each 4 h period (1-6: 1-4 h, 5-8 h, 9-12 h, 13-16 h, 17-20 h, 21-24 h) on training day 1 and 2 in the delta (0–5 Hz), theta (6–10 Hz), sigma (11–16 Hz) and beta (17–20 Hz) frequency bands during rapid eye movement (REM) sleep (A: learning, B: non-learning), slow wave sleep (SWS) (C: learning, D: non-learning) and wakefulness (wake) (E: learning, F: non-learning). Filled circles located at gridline intersections depicted in F indicate location of means plotted for each 4 h post-training period in each frequency band. Gradients between means were linearly interpolated to produce surface plots. Lights on were from periods 1-3, lights off from periods 4-6.
The opposite pattern of results was found for wakefulness (Figure 7C). There was a significant group by period interaction ($F(5, 85) = 3.26, p = 0.01$) on training day 1 and the learning group spent significantly more time awake in the 21–24 h period ($t(17) = 2.43, p = 0.03$). There was no significant difference in the percent duration of wake between learners and non-learners on the baseline day ($t(16) = -2.09, p = 0.052$), however, it should be noted that this effect did approach statistical significance.

**Power spectral analysis of the EEG.** There were no significant baseline group differences in delta ($t(17) = 1.14, p = 0.272$), sigma ($t(17) = 0.71, p = 0.490$), theta ($t(17) = 1.09, p = 0.295$) or beta ($t(17) = 0.76, p = 0.456$) power during SWS. Despite decreased SWS on the first training day, significant increases in sigma power were observed in the 21–24 h period for the learning group compared to the non-learning group (Figure 8C and D), but no significant differences were observed for delta, theta or beta. For sigma power on training day 1, a 2 x 6 (group x period) ANOVA revealed a significant group by period interaction ($F(5,85) = 3.37, p = 0.04$), and simple effects analysis revealed that the learning group had increased power compared to the non-learning group from 21–24 h only ($t(17) = 2.48, p = 0.02$). On the second training day during SWS, there was a more widespread difference across the theta, sigma and beta frequency bands in the first 4 h period (Figure 8C and D). There were significant group by period interactions in the theta ($F(5,80) = 3.85, p = 0.01$), sigma ($F(5,80) = 3.13, p = 0.001$), and beta frequency bands ($F(5,80) = 5.29, p = 0.002$), but not in the delta band ($F(5,80) = 1.33, p = 0.27$). Simple effects analysis revealed that the non-learning group had a significantly larger increase than the learning group from 1-4 h in the theta ($t(16) = 2.20, p = 0.04$) and the sigma ($t(16) = 2.18, p = 0.04$) but not the beta band.

There were no significant baseline group differences in delta ($t(17) = 1.00, p = 0.339$), sigma ($t(17) = 0.72, p = 0.481$), theta ($t(17) = 1.56, p = 0.136$) or beta ($t(17) = 1.02, p = 0.324$).
power during wakefulness. There were no significant group by period interactions on training day 1 or 2 during wake in the delta, theta, sigma and beta bands.

**Sleep spindle analysis.** During the same 21–24 h period on training day 1 when sigma power was greater in the learning group, sleep spindle density also increased in the learning group (Figure 9), indicating that the change in sigma power during this period may have been due to increases in sleep spindles. There was no significant baseline difference in sleep spindles between learners and non-learners (t(16) = 0.53, p = 0.60). A 2 x 6 (group x period) ANOVA revealed that spindle density changed across the six 4 h time periods on training day 1 as a function of learning condition (F(5,75) = 2.55, p = 0.03). Simple effects analysis revealed that the learning group had a significantly greater spindle density than the non-learning group during the 21–24 h period on training day 1 (t(15) = 2.95, p = 0.01). There were no significant main effects or group by period interaction on training day 2.

![Figure 9](image_url)

**Figure 9.** Mean change from baseline in sleep spindle density (#/min) during slow wave sleep (SWS) in each 4 h period (1-6: 1-4 h, 5-8 h, 9-12 h, 13-16 h, 17-20 h, 21-24 h) on training day 1 and 2. Learning rats (n=8) had a significantly greater increase compared to non-learning rats (n=10) in spindle density during the last 4 h post-training period (21–24 h) on training day 1 in SWS. Lights on were from periods 1-3, lights off from periods 4-6 indicated by the solid horizontal bar. Significant group difference from analysis of variance (ANOVA) followed by tests of simple effects indicated by * at p < 0.05.
Discussion

A discrete period of increased REM sleep was observed from 17–20 h following training (0900-1100 h) on a shuttle avoidance task in rats that learned the task. The increase was attributable to the number of REM episodes rather than their duration. We showed for the first time that increases in theta and beta power occurred during the RSW in learning rats. Learning-dependent changes in SWS also took place. SWS was decreased during the 21-24 h period that was characterized by increased sigma power and increased number of spindles per minute. Perhaps following the RSW, sleep spindles are involved in further memory consolidation processes. The finding of decreased minutes of SWS and increased wakefulness during this period might reflect reduced homeostatic sleep pressure from the preceding increase in REM sleep.

Previous studies have identified increased REM sleep from 9-12 h following avoidance learning (0900-1100h) using the same training regime as the current investigation (Smith and Lapp, 1986) but rats from a different source and a different light-dark schedule (lights on 0800 h, lights off 2000 h) were used. This may account for the shift in the RSW to 17-20 h observed in the present study. See Chapter 5 for a discussion of the study limitations. In agreement with previous studies (review Smith, 2003) the observed increase in REM sleep was of similar magnitude, persisted for a similar amount of time, and was not present on the second post-training day.

Theta activity is an EEG frequency band involved in learning (Buzsaki, 1989; Hasselmo, 2005; Lanfield, McGaugh and Tusa, 1972). Patterns of theta activity observed during exploration were reproduced during REM sleep (Louie and Wilson, 2001) suggesting reactivation of previously learned material. This may reflect a mechanism for sleep-dependent memory.
consolidation (Wilson and McNaughton, 1994). In humans, during training on a memory task, event-related increases in theta synchronization were positively correlated with good performance (Klimesch, 1999). Perhaps theta activity during REM sleep provides a pattern conducive to potentiating newly formed synaptic connections. Indeed, hippocampal LTP has been induced more effectively during post-training periods of increased REM sleep following training in the shuttle avoidance task (Bramham, Maho and Laroche, 1994).

Theta is suggested as the frequency that the hippocampus uses to communicate with other structures (Larson and Lynch, 1986; Larson, Wong and Lynch, 1986). The hippocampus is thought to have a time-limited role in the formation of memories (Frankland and Bontempi, 2005; Moscovitch et al., 2006) and the lasting storage of memory requires hippocampal-cortical transfer of information (Zola-Morgan and Squire, 1990). Increases in theta activity during REM sleep may represent hippocampal-neocortical communication that serves to reactivate newly formed memory traces in the neocortex. Increased sleep spindle activity may represent subsequent thalamocortical activity to induce LTP-like strengthening of cortical connections (Rosanova and Ulrich, 2005; Werk, Harbour and Chapman, 2005). As a result more enduring memory traces that become increasingly independent of the hippocampus will be formed. Thus, theta activity during REM sleep followed by spindle activity during SWS may represent two stages involved in consolidating the newly learned and behaviourally relevant aspects of a task.

Two-process models have been proposed whereby REM sleep and SWS together are involved in the consolidation of new learning (e.g., Giuditta et al., 2003). Based on human findings Smith et al. (2009) suggested that REM sleep may be involved in the consolidation of novel or complex learning, whereas Stage 2 sleep and specifically sleep spindles may be involved in the refinement of existing skills (Smith et al., 2009). Buzsaki (1989; 1996) suggested that in animals, acquisition occurs during gamma rhythms grouped by theta rhythms during exploratory
behaviour (Vanderwolf, 1969) and are replayed during sharp wave bursts involved in hippocampal-neocortical dialogue. During REM sleep, theta rhythms and fast frequency EEG in the beta/gamma range reactivate newly acquired memory traces. Sharp waves during SWS are involved in hippocampal-neocortical dialogue to transfer newly acquired memory traces to the cortex. A temporal correlation has been observed between hippocampal ripple (~200Hz) activity and neocortical sleep spindles during SWS (Siapas and Wilson, 1998) that may represent the transfer of information from the hippocampus to the neocortex. More recently, Buzsaki et al. (2005) have proposed that the slow oscillation (Steriade et al., 1993) may coordinate theta rhythms in the hippocampus and the activity of the sleep spindle.

The data from the current experiment support this model. They suggest that a 2-stage process is involved in memory consolidation during sleep whereby: 1) increases in theta activity occur during periods of increased REM, and 2) are followed by increases in sleep spindle activity during SWS (Figure 10).

The results from the present study provide evidence in the behaving rat that is consistent with a 2-stage model, but our correlational data do not provide a test of the model. The model predicts that the blockade of theta activity during REM sleep in the RSW or sleep spindles during SWS in the following period would impair subsequent performance. It further predicts that the second step is dependent on the outcome of the first. We are currently testing these predictions.

In conclusion, our results indicate that following avoidance learning, increases in REM sleep marked by intensified theta activity during the RSW are followed by increases in sleep spindles during SWS. Results provide some of the first evidence in behaving rats to support the complimentary 2-process models proposed by Smith et al. (2004) and Buzsaki (1989; 1996). First, REM sleep appears to be involved in hippocampal-neocortical transfer of information (as
indicated by increased theta activity), and second, SWS is involved in further refinement/stabilization in the neocortex (as indicated by increased sleep spindles). It is possible that a similar 2-stage process may occur in humans.

Figure 10. Schematic representation of the 2-stage model of sleep and memory as it applies to the two-way active avoidance paradigm. Increases in theta rhythms and rapid eye movement (REM) sleep occur in the latter part of the 24 h sleep-wake cycle, followed by increases in sleep spindles during non-REM sleep. Learning-dependent changes in REM sleep are hypothesized to represent hippocampal-neocortical dialogue, whereas learning-dependent changes in sleep spindles are thought to represent neocortical consolidation. The timing of the learning-dependent changes in sleep depends on the type of task and training regime.
Chapter 3

Increased GABAergic Activity in the Region of the Pedunculopontine and Deep Mesencephalic Reticular Nuclei Reduces REM Sleep and Impairs Learning in Rats

Electroencephalographic (EEG) recordings in humans from scalp electrodes reveal changes following different types of learning (e.g., procedural and declarative) that occur during different stages of sleep (Fogel, Smith and Cote, 2007; for review see Walker, 2005). In nearly all mammalian species, sleep is typically categorized into rapid eye movement (REM) and non-REM sleep. In humans, non-REM sleep is typically further subdivided into: stage 1 (sleep onset), stage 2, characterized by sleep spindles and stages 3 and 4 (collectively called slow wave sleep; SWS) characterized by delta (or slow wave) activity.

REM sleep is involved in memory consolidation in both humans and animals (for review see Smith, 2003; Smith, 1996; Walker, 2005). While there is a compelling body of evidence to support the role of REM sleep in memory consolidation in rodents, there is little consensus on the exact role that REM sleep may play in memory consolidation in humans (Smith, 2001; Stickgold and Walker, 2005a; Stickgold and Walker, 2005b; Vertes and Siegel 2005a; Vertes and Siegel, 2005b; Walker, 2005). It is a slow process that may involve the transfer of newly learned information from the medial temporal lobe to the neocortex (Frankland and Bontempi, 2005; Moscovitch et al., 2006; Zola-Morgan and Squire, 1990) that serves to transform initially labile memories into more enduring memories. Learning-dependent changes in REM sleep have been observed (Fogel, Smith and Cote, 2007; Smith and Lapp, 1991) and REM sleep deprivation impairs memory in humans (Tweed et al., 1999) and animals (Hennevin, Hars, Maho and Bloch, 2005).

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1995; Legault, Smith and Beninger, 2004; Pearlman, 1979). In rats, post-training periods termed REM sleep windows (RSWs), characterized by increased REM sleep, that typically last for about four hours are observed after training on a number of tasks (for review see Smith 1985; 1996; 2003). The intensity of training and the specific task affects the timing of the RSW (Smith, Young and Young, 1980). If REM sleep is disrupted during these periods, subsequent performance impairments are observed (Smith and Kelly, 1988; Smith and Lapp, 1986). Thus, RSWs are discrete periods marked by enhanced REM sleep and are thought to be a time when sleep-dependent memory consolidation occurs.

RSWs have been identified in rats for a number of tasks (Smith, Conway and Rose, 1998; Smith and Rose, 1997; Smith and Wong, 1991) including two-way active avoidance (Fogel, Smith and Beninger, 2009; Smith and Lapp, 1986; Smith, Young and Young, 1980). Rats that learned the task had increased REM sleep from 17-20 h following training on the first day compared to rats that did not learn the task (Fogel, Smith and Beninger, 2009). The theta rhythm (6-10 Hz) characterizes the EEG of wakefulness during voluntary behaviour (Vanderwolf, 1969) and is particularly prominent in the EEG of REM sleep in rodents. Theta rhythms may represent hippocampal-neocortical dialogue (Buzsáki, 1996; Hasselmo, 2005; Landfield, McGaugh and Tusa, 1972), and sleep spindles, which characterize non-REM sleep may be an ideal mechanism for the induction of long-term potentiation-like changes in the neocortex (Steriade and Timofeev, 2003). Fogel, Smith and Beninger (2009) found that theta power increased during the RSW, followed by increases in sleep spindles. These findings suggest that sleep-dependent memory consolidation may involve both SWS and REM sleep.

The pedunculopontine nucleus (PPN) participates in the generation and maintenance of REM sleep (Pace-Schott and Hobson, 2002; Rye, 1997). The PPN is located in the brainstem tegmentum and is strongly interconnected with the basal ganglia, being reciprocally connected to
the globus pallidus (Mena-Segovia et al., 2004) and projecting to the substantia nigra zona compacta and the subthalamic nucleus (Pahapil and Lozano, 2000). With respect to sleep generating mechanisms, the cholinergic cells of the PPN have generated the greatest interest. The rostral aspect of the PPN is a heterogeneous group of cells (Rye, 1997) with 70% of the cells being glutamatergic (Clements and Grant, 1990). The more caudal aspect of the PPN (pars compacta) contains roughly 90% of the cholinergic neurons (Rye et al., 1987). In total, the rat PPN contains approximately 2,200 cholinergic neurons. The recently revised reciprocal-interaction model (Pace-Schott and Hobson, 2002) originally described by Hobson, McCarley and Wyzinski (1975) proposes that two groups of cells (REM-on and REM-off) with reciprocal inhibitory circuits interact to generate non-REM and REM states. Cells in the PPN have been differentiated based on firing rates across states of wakefulness. Serotonergic REM-off cells located in the raphe nuclei and noradrenergic cells of the locus coeruleus are most active during wake and decrease their activity as non-REM sleep progresses. They exert an inhibitory influence on REM-on cells, and reciprocally to themselves. The excitatory cholinergic REM-on cells of the PPN/laterodorsal tegmental nucleus (LDT) and the glutamatergic cells of the brainstem reticular formation fire preferentially during REM sleep (Datta, 1995; Steriade et al., 1990). They exert an inhibitory influence on REM-off cells and reciprocally to themselves. Ascending PPN/LDT pathways to the basal forebrain via the thalamus modulate cortical arousal, descending pathways to the pontine reticular formation generate ponto-geniculo-occipital waves and muscle atonia. Thus, the PPN is the last common junction for the expression of the definitive electrophysiological features of REM sleep. Receptors for the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) found in the PPN (along with receptors for serotonin, adenosine and norepinephrine) may be involved in REM sleep regulation (Steriade and McCarley 1990). Thus, the GABA\textsubscript{B} agonist baclofen significantly reduced REM sleep (Ulloor et
al., 2004), whereas serotonin, adenosine and norepinephrine in the PPN had no significant effect on REM sleep regulation (Datta et al., 2003). It has been found that activation of GABA_B but not GABA_A or GABA_C receptors in the PPN reduced activity of REM-on cells (Ulloor et al., 2004). These cells are thought to be cholinergic (Jia et al., 2003), projecting widely throughout the brainstem and forebrain (Datta, 1995; Rye, 1997). A recent study (Sapin et al., 2009) has identified a population of GABAergic neurons adjacent (dorsomedial) to the PPN in the deep mesencephalic reticular nucleus (DpMe) that are thought to control REM sleep similarly to the PPN. The PPN has also been implicated in incentive learning; e.g., performance on the two-way active avoidance task is impaired following electrolytic lesions of the PPN region (Satorra-Marin et al., 2001). Avoidance learning is dependent on other structures such as the amygdala (Ashford and Jones, 1976; Schutz and Izquierdo, 1979), presumably due to the emotional context. Using contextual fear conditioning, it was found that theta activity between the amygdala and hippocampus was synchronized 1 day after training, but not at shorter (2 h) or longer (30 day) intervals (Narayanan et al., 2007).

The present study investigated sleep-dependent memory consolidation by increasing inhibition in the PPN/DpMe by infusing the GABA_B agonist baclofen to increase inhibition in REM-on cells during the previously observed RSW (Fogel, Smith and Beninger, 2009). It was hypothesized that reduced REM sleep would be observed during the RSW in animals receiving baclofen infusions, and that memory would be impaired in these animals.

**Experimental Procedures**

Housing, behavioural testing and EEG recording techniques were identical to those described by Fogel, Smith and Beninger (2009) and are presented in abbreviated form here. Twenty-five male Sprague-Dawley rats (Charles River, St. Contstant, QC) individually housed with *ad-libitum* food and water and kept on a 12-h light-dark cycle (lights off at 2300 h) weighed
250-300 g at the beginning of the study and were implanted with four epidural EEG and two electromyogram (EMG) electrodes. Guide cannulae (Plastics One: 5.0 mm long, 0.46 mm outer diameter, 0.24 mm inner diameter, 26 guage) were implanted bilaterally into the PPN/DpMe region according to coordinates obtained from Paxinos and Watson (1998): from bregma posterior 7.8 mm, lateral 1.8 mm from the midline and 5.0 mm ventral from the surface of the skull. The cannulae were anchored to the skull with jeweller’s screws and dental acrylic. Stylets (Plastics One: 5.0 mm long, 0.20 mm diameter) were inserted into the cannulae until injections were made.

After 14 days of recovery, 3 days of acclimatization, and 24 hours of baseline recording, animals were trained on the two-way avoidance task for 100 trials (50 trials/day) and re-tested for 25 trials on day 3 starting at 0900 h and ending by 1100 h. Prior to training, a 5-min acclimatization was allowed whereby the rat could roam freely from one compartment to the other. Each trial consisted of a 10-s interval when the compartment containing the rat was illuminated and a tone was presented. Following the 10 s interval, a 1.5 mA footshock (20 s maximum) was administered. The light, tone and footshock were terminated upon crossing. If the rat escaped during the 10 s interval, no shock was administered and the light and tone terminated upon crossing. Each trial was separated by a 10 to 20-s inter-trial interval. Sleep was recorded for 22-24 h after training on both training day 1 and 2 beginning between 1000 and 1100 h. EEG was stage scored as REM, slow wave sleep (SWS) or wake in 30-s epochs. Epochs were scored as wake when the EEG was high frequency, low voltage and desynchronized with high and variable EMG amplitude. Epochs were marked as SWS when the EEG was high amplitude, slow frequency and marked by periodic sleep spindles with reduced amplitude EMG compared to wakefulness. REM was identified when low voltage theta rhythms characterized the EEG and EMG amplitude was lowest. All stage and event scoring was made blind to the recording day and
study condition. Sleep spindles were automatically detected using a computerized algorithm (Prana, Phi Tools, Strasbourg, France). Sleep spindles were scored when the EEG from 11-16 Hz was greater than 100 µV in amplitude, lasting a minimum of 0.15 s, maximum duration of 3 s and a minimum inter-spindle interval of 0.25 s. Spindles identified with an inter-spindle interval of less than 0.25 s were merged and counted as a single spindle. Rats in the learning group (n=8) avoided footshock on 60% of the last 20 test trails. The remaining rats (n=8) were assigned to the non-learning group. No rats in the baclofen group (n=8) performed above criterion. The RSW was identified in a previous experiment (Fogel, Smith and Beninger, 2009) to be from 17-20 h following training day 1. Thus, the same training regime (50 trials/day for two days) was used here so that baclofen injections could be made during the same known RSW. Drug infusions were not made on the training day 2 as the 17-20 h period does not correspond to a known RSW. Earlier experiments identified the RSW for this training regime at 9-12 h after training (Smith and Lapp, 1986), but rats from a different source, different light cycle and time of testing were used in the more recent (Fogel, Smith and Beninger, 2009) and present study. The GABA_B receptor agonist baclofen (Sigma-Aldrich, Canada) was infused into the PPN/DpMe region at the optimal dose (1.5 nmol) shown previously (Ulloor et al., 2004) to reduce REM sleep for approximately 4 h. Infusions were made during the RSW on training day 1 (17 h post-training, at 0300 h) via the implanted cannulae using an injector (Plastics One: 7.0 mm long, 0.20 mm outer diameter, 0.10 mm inner diameter, 33 guage) attached by tubing to a microsyringe mounted on an infusion pump. The drug (0.375 µg per side) was infused via the implanted cannulae at 17 h post-training on training day 1 over 30 seconds in a volume of 0.5 µL of phosphate buffered 0.9% saline per side. The remaining rats received an infusion of saline (0.5 µL per side) into the PPN/DpMe region at the same time as the baclofen group. The injection cannulae were kept in place for 2 min after the infusion to allow for diffusion. Following experimental testing, rats were
euthanized, perfused with 10% formal-saline and the brains were placed in 10% formal-saline bath prior to sectioning. Brain tissue was sectioned on a sliding microtome at 60 microns thick. Coronal sections taken every 360 microns were mounted on gelatin-coated glass microscope slides and stained with Cresyl Violet (Figure 11). All procedures were approved by the Trent University ethics review board.

Figure 11. Photomicrograph of a 60 micron thick coronal section, -7.8 mm from bregma. Both the location of the guide and injector canulas are visible. Injector tip is within the PPN/DpMe region of the brainstem. Solid horizontal bar represents 1.0 mm.
Cannula tips were verified (Figure 12) according to diagrams from Paxinos and Watson (1998). All rats in the baclofen group had at least one canula tip within the PPN/DpMe, and were included in the behavioural and sleep architecture data analyses. Damage to the primary visual cortex and the boundary areas between the superior and inferior colliculus the diameter of the guide canula (0.46 mm outer diameter) was observed due to the implanted cannula. Cannulas did not appear to displace the fourth ventricle. One rat in the saline learning group had both canulae tip placements outside the boundaries of the PPN and DpMe. The canula tips for this rat were however within 0.5 – 1.0 mm of the target area. Given the volume used here (0.5 µL) and infusion rate (1 µL/min) it is expected that the diffusion area would be approximately 1 mm (Myers, 1966). Mixed-design analysis of variance (ANOVA) was used to analyze the data (see below) with and without the saline rat with both injector tips outside the PPN/DpMe. Excluding the saline rat with both placements outside the PPN/DpMe did not affect the outcome of the main interaction effects, thus, all saline rats were included in all data sets.

Figure 12. Coronal section, -7.8 mm from bregma identifying the location of injector tips for baclofen (grey circles), saline learning (black circles) and saline non-learning rats (unfilled circles). Figure adapted from Paxinos and Watson (1998). The PPN/DpMe is indicated by the shading. A single injector tip location may represent more than one placement.
Statistical Analysis

The sleep architecture and spindle data were binned into the 4-hour periods, aligned according to lights on (1100 h) and labeled periods 1–6 for each training day as follows: 1: 1100 to 1500 h, 2: 1500 to 1900 h, 3: 1900 to 2300 h, 4: 2300 to 0300 h, 5: 0300 to 0700 h, 6: 0700 to 1100 h. All sleep stages were adjusted for baseline and expressed as the percent total recording time within each 4-hour period, and sleep spindles by the total duration in minutes of SWS sleep to yield a measure of spindle density (spindles/minute).

Behavioural data were analyzed using mixed design 3 x 3 (group by day) ANOVA. For the percentage of correct avoidances, a significant interaction was followed up by tests of simple effects of day for each group to test for significant changes in performance over training and retest days. Sleep architecture data were analyzed using a mixed design 3 x 6 (learning group by period) ANOVA. A significant interaction was followed up by tests of simple effects of group at each period, and independent t-tests. Spindle density was analyzed using the same approach. When the assumption of homogeneity of variance was violated, the degrees of freedom were corrected using the Huynh-Feldt correction factor. Poor EEG recording quality resulted in missing data for one rat in the saline group for sleep architecture and sleep spindle analyses. The case with missing data was excluded from statistical analyses.

Results

Behavioural Data

Rats in the Saline condition were easily distinguished into learners and non-learners (Figure 13). There were no rats in the baclofen group that learned the task (Figure 13 and Figure 14). The percentage of correct avoidances significantly changed over the three days of training and testing as a function of group (F(4,42) = 7.49, p < 0.0001). Tests of simple effects of day for each group revealed that the saline learning group significantly increased the number of
avoidances over the three days of testing ($F(2,14) = 13.09, p < 0.001$), whereas the saline non-learning group and the baclofen group had no significant change in correct avoidances over the three testing days.

**Figure 13.** Number of correct avoidances for each rat on the retest day in the saline learning (black bars; $n = 8$) saline non-learning (unfilled bars; $n = 8$) and baclofen groups (grey bars; $n = 8$). Dashed horizontal line indicates the 60% criterion. Data were rank-ordered by rat in descending order.

**Figure 14.** Two-way shuttle performance (percentage (+/- SEM) of correct avoidances) in the saline learning ($n = 8$), saline non-learning ($n = 8$) and baclofen ($n = 8$) groups on the two training days and the re-test. Dashed horizontal line represents 60% correct performance criterion. * significant ($p < 0.05$) simple effects of days for the saline learning group following significant day by group interaction in analysis of variance.
Sleep Architecture Data

REM Sleep. Rats in the learning condition had increased REM sleep during the RSW (17-20 h), whereas the baclofen condition had significantly less REM during the RSW on training day 1 and increased REM from 17-20 h on training day 2 (Figure 15A). There was no baseline group difference (F(2,20) = 1.73, p = 0.20). A 3 x 6 (group by period) ANOVA for REM sleep on training day 1 revealed a significant group by period interaction (F(10,100) = 3.75, p < 0.001). Simple effects analyses of group at each period revealed that the groups were significantly different in the 17-20 h period only (F(2, 22) = 13.37, p < 0.0001). Post-Hoc tests revealed that the saline learning group had significantly more REM sleep than either the saline non-learning group (t(13) = 2.27, p = 0.041) or the baclofen group (t(14) = 4.55, p < 0.0001). The baclofen group had significantly less REM sleep than the non-learning group (t(13) = 2.99, p = 0.010).

There was also a significant group by period interaction on the second training day (F(10,100) = 2.66, p = 0.006). Simple effects analysis revealed that the groups differed in the 17-20 h period only (F(2,22) = 4.72, p = 0.021). The baclofen group had more REM during the 17-20 h period (t(13) = 3.03, p = 0.010) than the saline non-learning, but not the saline learning group. The saline learning group did not differ significantly from the saline non-learning group.

SWS sleep. There was no baseline group difference (F(2,20) = 0.25, p = 0.78). A 3 x 6 (group by period) ANOVA for SWS sleep on training day 1 revealed that the amount of SWS changed over the course of the six 4-h periods irrespective of group (Figure 15B), indicated by a significant main effect of period (F(5,100) = 23.75, p < 0.001) and a non-significant effect of group (F(2,20) = 3.44, p = 0.052). However, there was no significant group by period interaction.

On training day 2, there was a significant main effect of period (F(5,100) = 20.77, p < 0.001) and a significant main effect of group (F(2,20) = 4.23, p = 0.029), but no significant group by period interaction.
Figure 15. Mean difference scores from baseline to training day 1 and 2 for the duration of rapid eye movement (REM) sleep (A), slow wave sleep (SWS) (B) and wakefulness (wake) (C) expressed as a percentage of total recording time (%TRT) within each 4-hour post-training recording period (1-6: 1-4 h, 5-8 h, 9-12 h, 13-16 h, 17-20 h, 21-24 h, respectively) in the saline learning (n = 8), saline non-learning (n = 7) and baclofen (n = 8) groups. Lights on were from periods 1-3, lights off from periods 4-6 indicated by the filled horizontal bar. * significant (p < 0.05) simple effect of group following significant period by group interactions in separate analyses of variance (ANOVA) for training days 1 and 2.
**Wake.** There was no baseline group difference (F(2,20) = 0.05, p = 0.95). A 3 x 6 (group by period) ANOVA for wake on training day 1 revealed that the amount of wakefulness changed over the course of the six 4-h periods irrespective of drug condition (Figure 15C), indicated by a significant effect of period (F(5,100) = 11.68, p < 0.001). There was no significant effect of group, or group by period interaction. On training day 2, there was a significant effect of period (F(5,100) = 15.71, p < 0.001) but no significant effect of group or group by period interaction.

**Sleep Spindle Data.** Sleep spindles during SWS increased in the saline learning group but not in the saline non-learning or baclofen groups in the last 4 to 8 hours on training day 1 (Figure 16). There was no baseline group difference (F(2,20) = 1.29, p = 0.30). A 3 x 6 (group by period) ANOVA revealed a significant interaction indicating that sleep spindles varied over period as a function of group (F(10,100) = 1.94, p = 0.048). Follow-up simple effects analysis identified group differences in sleep spindles in the 17-20 h (F(2,20) = 6.72, p = 0.012) and 21-24 h periods (F(2,20) = 3.93, p = 0.048). Post-hoc tests revealed that for the 17-20 and 21-24 h periods, the saline learning group had significantly more sleep spindles than the saline non-learning (t(13) = 3.01, p = 0.01, t(13) = 2.51, p = 0.026, respectively) and baclofen group in the 17-20 h period (t(14) = 2.93, p = 0.01) and a non-statistically significant difference in the 21-24 h period (t(14) = 2.13, p = 0.052). The saline non-learning group and the baclofen group did not differ significantly from one another. No significant effects were observed for training day 2.
Figure 16. Mean change from baseline in sleep spindle density (#/min) during slow wave sleep (SWS) within each 4-h post-training period (1-6: 1-4 h, 5-8 h, 9-12 h, 13-16 h, 17-20 h, 21-24 h, respectively). Saline learning rats (n = 8) had a significantly greater increase over baseline compared to saline non-learning rats (n = 7) and the baclofen group (n = 8) in spindle density during the last two 4-h post-training periods (17-20 h, 21–24 h) on training day 1. Lights on were from periods 1-3, lights off from periods 4-6 indicated by filled horizontal bar. * significant (p < 0.05) simple effect of group following significant period by group interaction on training day 1. The saline learning group differed significantly from pairwise comparisons between saline non-learning and baclofen groups in period 5 and between the saline non-learning group in period 6.

Discussion

REM sleep increases were observed during the RSW in the saline learning group compared to the saline non-learning and baclofen groups. Infusions of the GABA<sub>B</sub> agonist baclofen into the region of the PPN/DpMe decreased REM sleep and impaired subsequent memory performance. Infusions were made at a time when learning-dependent increases in REM sleep were previously observed (Fogel, Smith and Beninger, 2009). Rats receiving saline infusions were distinguishable into learners and non-learners, whereas none of the rats that received baclofen was able to learn the task. The changes in REM sleep appear to have had a
(statistically non-significant) trend on the percent duration of SWS and wake. There were no group by time period interactions on either training day 1 or 2 in SWS or wake. There were significant group effects indicating that the drug infusion while reducing REM sleep, increased wakefulness over a more protracted time period. While not statistically significant, the learning group tended to spend less time in SWS when REM sleep was significantly increased.

The PPN/DpMe has been identified as an important structure involved in the regulation of REM sleep (Rye, 1997; Sapin et al., 2009). The present study showed that injection of baclofen into the region of the PPN/DpMe 17 h following training on an avoidance task decreased the duration of REM sleep in the 17-20 h period suggesting that REM-on cells were inhibited.

The duration of REM sleep increased during the 4-h period from 17-20 h following learning on a two-way avoidance task (Fogel, Smith and Beninger, 2009), the RSW for this task. This increase was replicated in the present study, the saline learning group having a significantly more REM sleep than the saline non-learning group exclusively during the 17-20 h period after training on training day 1. The present study showed for the first time that impaired shuttle performance was observed in rats that had reduced REM sleep with injection of a GABA$_B$ receptor agonist into the region of the PPN/DpMe during the RSW. Although baclofen was not injected during other 4-h periods following training and the possible mnemonic effects of such injections remain unknown, the present finding is consistent with the hypothesis that REM sleep during the RSW is involved in memory consolidation (for review see Smith, 2003). Previous studies using a similar training regime have identified increased REM sleep on the first training day, but not on the second day (Fishbein, Kastaniotis and Chattman, 1974; Fogel, Smith and Beninger, 2009; Smith and Lapp, 1986). Furthermore, REM sleep deprivation (using the “flower-pot” technique) during the RSW impairs memory (for review see Smith, 1996) and 4-hours of REM sleep deprivation at intervals outside the RSW does not significantly impair performance...
Systemic injections of the cholinergic antagonist, scopolamine impaired performance on the radial arm maze task in a dose-dependent manner when injections were given during the RSW, but not outside the RSW (Legault, Smith, and Beninger, 2004). When REM sleep deprivation occurs at all other post-training times except for the RSW, subsequent memory performance is not significantly impaired compared to normally rested controls (Smith and Butler, 1982). Despite further training on the task, rats prevented from having REM sleep during this period were not able to improve performance on subsequent training days. The present study shows that inhibition of REM sleep by injection of a GABA\(_B\) agonist into the region of the PPN/DpMe during the RSW was associated with impaired memory. Taken together these findings suggest that the RSW is a critical period for the enhancement of previously learned material. If learning-related increases in REM sleep do not occur during this time, prolonged impairment is observed. Importantly, despite increased REM sleep during the 17-20 h period on training day 2, no increase in performance was observed suggesting that this effect may reflect a REM rebound (Dement, 1960) not related to training. This suggests that the RSW is a period that is critical for memory consolidation to take place. If the opportunity to consolidate new learning is missed, even if increased REM is observed at the same time the following day, there is no subsequent improvement in performance. Similar results have been observed in humans (Walker et al., 2002).

In the present study, sleep spindles increased during the 17-20 h and the 21-24 h period following training on training day 1 in the saline learning group compared to the saline non-learning or baclofen groups. Increased sleep spindles were observed in learning compared to non-learning rats in a previous experiment (Fogel, Smith and Beninger, 2009) during the 21-24 h period. Sleep spindles have been implicated in memory consolidation in humans (Clemens et al., 2002).
Sleep spindles have been suggested as an ideal mechanism for long-term potentiation (LTP) in the neocortex (Steriade and Timofeev, 2003), which has been demonstrated in *in vitro* (Rosanova and Ulrich, 2005) and *in vivo* (Werk, Harbour and Chapman, 2005) experiments. It cannot be determined from the present experiment if the baclofen infusion affected sleep spindle production as well as increases in spindles were observed only in rats that learned the task. None of the rats that received the baclofen infusion was able to learn the task, thus a learning-dependent change in spindles would not be expected in this condition. Future research could dissociate learning-dependent changes in sleep spindles from the learning-dependent changes in REM sleep.

Neurodegeneration of cells in the PPN is observed early in Parkinson’s disease (Braak et al., 2003) before cell death is observed in the substantia nigra and before signs of the disease are expressed. REM sleep disturbances are common in Parkinson’s disease and one of the early risk factors for Parkinson’s disease is the development of REM behaviour disorder (Gagnon et al., 2006; Iranzo, Molinuevo and Santamaria, 2006). Cell death in the PPN is thought to be involved in the development of REM behaviour disorder (Rye, 1997). REM behaviour disorder is characterized by the loss of normal muscle paralysis in the major muscle groups normally observed during REM sleep in humans. The loss of paralysis results in the often-violent enactment of dreams occurring during REM sleep. Parkinson’s disease eventually develops in about 40% of all patients diagnosed with REM behaviour disorder (Iranzo et al., 2006; Schenck, Bundlie, Mahowald, 1996). The basal ganglia project heavily to the PPN and one recent review proposes that it may be possible to consider the basal ganglia and PPN as a single functional unit (Mena-Segovia et al., 2004). Given the PPN’s role in the regulation of REM sleep, and it’s connectivity with the basal ganglia, it is possible that disrupted REM sleep in Parkinson’s disease
may contribute to memory deficits observed in Parkinson’s patients. In the current study, the REM-on cells of the PPN/DpMe were putatively inhibited by increasing GABAergic activity. Learning impairments were also observed in drug infused rats. Neurodegeneration of PPN cells in Parkinson’s and REM behaviour disorder may be responsible for a similar loss of cholinergic input to the ascending reticular activating system, and descending input to pontine regions resulting in disrupted REM sleep and characteristic loss of REM atonia. Disruption to REM sleep from damage to cholinergic cells of the PPN may contribute to cognitive impairments observed in Parkinson’s disease. The results of the present study are consistent with this hypothesis, however we do not have information about the EMG muscle tone in the baclofen treated group from the present experiment.

The results of the present study suggest that both SWS and REM sleep are involved in sleep-dependent memory consolidation during discrete post-learning periods. REM sleep may be a period whereby hippocampal-neocortical dialogue is involved in the transfer of newly learned memories from the hippocampus to the neocortex. Non-REM sleep may be necessary for subsequent refinement and neocortical plasticity to occur. Future studies could investigate whether blocking sleep spindle activity during SWS impairs subsequent performance as well to determine if both processes are necessary for normal learning.
Chapter 4
Too Much of a Good Thing? Elevated Baseline Sleep Spindles Predict Poor Avoidance Performance in Rats

The functions of sleep remain largely enigmatic, but one of the dominant hypotheses is that sleep plays an important role in the formation of new memories. Newly encoded memories are initially in a labile state. The process by which memories are transformed into a more enduring state is called consolidation and depending on the type of learning is thought to take hours (Mednick, Nakayama and Stickgold, 2003; Korman et al., 2007), days (Scoville and Milner, 1957; Kim and Fanselow, 1992), weeks (Zola-Morgan and Squire, 1990), months, or even years (Haist et al., 2001). Since one of the earliest investigations by Jenkins and Dallenbach (1924) it has been known that sleep can produce gains in performance without additional practice for which the simple passage of time cannot account (for review see Born, Rasch, and Gais, 2006; Diekelmann, Wilhelm and Born, 2009; Peigneux et al., 2001; Peigneux and Smith, in press; Walker and Stickgold, 2006).

Sleep spindles may provide an electrophysiological signature of a potential mechanism for sleep-dependent memory consolidation. In humans, the density of sleep spindles appears to be consistent for any individual from night-to-night (De Gennaro et al., 2005; Gaillard and Blois, 1981; Silverstein and Levy, 1975); it has been remarked that inter-individual characteristics in sleep spindle density are reliable enough to serve as an “electrophysiological fingerprint” (DeGennaro et al., 2005). The functional significance of sleep spindles has not been investigated until recently. Inter-individual differences in sleep spindle density may be a physiological marker for intellectual ability as measured by aptitude tests that typically use the age-normed intelligence quotient (IQ). Sleep spindle density has been found to be positively correlated with performance on the Multidimensional Aptitude Battery – II (Fogel et al., 2007), the Raven’s progressive
matrices test (Bodizs et al., 2005) and the Wechsler Memory Scale (Schabus et al., 2006). Sleep spindle density has been found to be positively correlated with verbal memory retention (Briere et al., 2000; Gais et al., 2002; Clemens, Fabo, and Halasz, 2005) and visuospatial memory (Clemens, Fabo, and Halasz, 2006). Schabus et al. (2004) found that individuals with increased spindle activity during Stage 2 sleep had increased recall from pre-sleep to post-sleep testing, whereas those who did not have increased spindle activity did not improve on the task. Learning-dependent increases in sleep spindle density have been observed following motor procedural learning (Fogel and Smith, 2006; Fogel, Smith and Cote, 2007) and are thought to be a putative marker of consolidation.

Specific characteristics of sleep spindles are related to below normal IQ. Sleep spindles that were higher in amplitude and longer in duration, termed “extreme spindles” were identified in 70-80% of developmentally delayed children (Gibbs and Gibbs, 1962; Winfield et al., 1955; Oka, 1970). Bixler and Rhodes (1968) identified a similar pattern in adolescents, and suggested that extreme spindles may result from lowered reciprocal inhibition in thalamocortical neurons.

In rats, it has been demonstrated that induction of long-term potentiation (LTP) results in increased reliability of evoked sleep spindles (Werk, Harbour and Chapman, 2005) and conversely, that sleep spindle-like activity can produce LTP in preparations of rat somatosensory cortex in vitro (Rosanova and Ulrich, 2005). In vivo studies have demonstrated that sleep spindle density increased following reward learning in rats; when the reward was available noncontingently, there was no subsequent change in sleep spindles (Eschenko et al., 2006).

Critical and discrete (about 4 hour) post-learning periods marked by increased REM sleep (termed REM sleep windows) have been indentified in the rat (for review see Smith, 1985; 1996; 2003). Recently, we have identified learning-related increases in sleep spindle density (from 21 to 24 hours) following avoidance learning in rats that occurred immediately following the REM
sleep window (from 17 to 20 hours; Fogel, Smith and Beninger, 2009). These results suggest that sleep-dependent memory consolidation may occur in (at least) two sequential steps and provide support for the sequential hypothesis, initially proposed by Giuditta (first described by Giuditta, 1977; 1985; see Giuditta, 2003 for a recent review of the sequential hypothesis).

Pairs of hippocampal cells that are coactive during wake have been shown to be reactivated during subsequent sleep (Palvides and Wilson, 1989; Wilson and McNaughton, 1994). During SWS, bursts of high frequency activity (termed hippocampal ripples) originating from CA1 region pyramidal cell activity in the hippocampus provide ideal conditions for long-term synaptic plasticity to occur (Buzsáki, 1987; Bliss and Coolingridge, 1993). Siapas and Wilson (1998) demonstrated that hippocampal ripples are temporally related to sleep spindle activity. Hippocampal ripples precede cortical spindles by less than 1 second, whereby spindle and ripple onset times are highly correlated with one another. Baseline slow wave activity groups spindle and ripple activity (Molle et al., 2009). These results suggest that sleep spindles may be involved in hippocampal-neocortical dialogue taking place during SWS, and may be an important mechanism or identifiable feature in the electroencephalogram (EEG) related to sleep-dependent memory consolidation. The present study investigated the relationship between baseline sleep architecture and sleep spindles with shuttle avoidance performance to identify sleep EEG predictors of the ability to learn. Based on our previous results (Fogel, Smith and Beninger, 2009) it was predicted that sleep spindles during the 21 to 24 hour baseline period may predict avoidance performance.

**Methods**

The data reported here were collected during the conduct of the experiments reported by Fogel, Smith and Beninger (2009) and Fogel, Smith and Beninger (in press). In total, 37 male Sprague-Dawley rats (Charles River, St. Constant, QC) were housed with *ad-libitum* food and
water and kept on a 12-hour light-dark cycle (lights off at 2300 h), weighed 250-300 g at the time of surgery and were implanted with four epidural EEG and two electromyogram (EMG) electrodes. Rats in the learning group (n = 16) avoided footshock on at least 60% of the last 20 test trails on the re-test one day after training day 2. The remaining rats (n = 21) were designated as the non-learning group. Surgery, acclimatization, behavioural training, testing, EEG recording and analysis were the same as previous studies (Fogel, Smith and Beninger, 2009; in press). See Chapters 2 and 3 for detailed methodology. All procedures were approved by the Trent University ethics review board.

**Statistical Analysis**

The sleep architecture and spindle data were binned into the same 4-hour periods, aligned according to lights on (1100 h). All sleep stages were expressed as the percent total recording time within each 4-hour period, and sleep spindles by the total duration in minutes of SWS sleep to yield a measure of spindle density (spindles/minute). Rats with poor quality EEG recordings that could not be reliably stage scored (n = 1 non-learning, n = 1 learning) or analyzed for spindle detection (non-learning: n = 4; learning: n = 2) were excluded from statistical analyses.

Behavioural data were analyzed using mixed design 2 x 3 (group by block) analysis of variance (ANOVA). For the percentage correct avoidances, a significant interaction was followed up by tests of simple effects of day for each group to test for significant changes in performance over blocks of trials. Sleep architecture and spindle density data were analyzed using a mixed design 2 x 6 (learning group by period) ANOVA on baseline recordings. Pearson’s Correlation Coefficients were calculated between re-test avoidance performance and each baseline 4-hour period for REM, SWS, wake and spindle density. For each set of variables, the p-level was Bonferroni corrected for the family-wise number of comparisons (i.e., for six comparisons, p <
0.0083). A similar approach was used to assess the correlation between re-test avoidance performance and the change in sleep spindle density from baseline to the first training day.

**Results**

**Behavioural Data**

Rats were clearly distinguished as either learning or non-learning rats at re-test according to previously established criteria (Fogel, Smith and Beninger, 2009). Learning rats increased avoidance responses over the training and testing sessions, whereas the non-learning rats did not (Figure 17). A 2 x 3 (group x day) ANOVA for avoidance responses revealed a significant group by day interaction ($F(2, 70) = 13.82, p < 0.0001$). Avoidance responses significantly increased over the training and testing days as revealed by follow-up simple effects repeated measures ANOVAs for the learning group ($F(2, 30) = 23.43, p < 0.0001$) but no significant change was observed in the non-learning condition ($F(2, 40) = 0.32, p = 0.73$).

![Figure 17](image-url) Mean percent correct avoidances at training day 1, 2 and re-test. Rats performing above 60% criterion (dashed horizontal line) at re-test are indicated by filled circles ($n = 16$), and unfilled circles for non-learning rats ($n = 21$). Error bars represent standard error of the mean.
**Sleep Architecture Data**

There were no significant group differences between the learning and non-learning groups at baseline for REM, SWS or wake across the six 4-hour periods (Table 2).

**Table 2.** Means (M) and standard deviations (SD) of percent total recording time for each 4-hour period on baseline categorized by REM, SWS, and wake in learning (n=15) and non-learning rats (n=20).

<table>
<thead>
<tr>
<th>Period</th>
<th>Learning</th>
<th></th>
<th>Non-Learning</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Baseline REM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4h</td>
<td>0.71</td>
<td>0.51</td>
<td>1.55</td>
<td>1.95</td>
</tr>
<tr>
<td>5-8h</td>
<td>5.39</td>
<td>4.37</td>
<td>5.41</td>
<td>2.96</td>
</tr>
<tr>
<td>9-12h</td>
<td>5.18</td>
<td>3.48</td>
<td>5.79</td>
<td>3.15</td>
</tr>
<tr>
<td>13-16h</td>
<td>9.28</td>
<td>3.81</td>
<td>9.28</td>
<td>3.88</td>
</tr>
<tr>
<td>17-20h</td>
<td>12.45</td>
<td>3.64</td>
<td>10.29</td>
<td>4.95</td>
</tr>
<tr>
<td>21-24h</td>
<td>3.78</td>
<td>3.55</td>
<td>5.61</td>
<td>4.80</td>
</tr>
<tr>
<td>Total</td>
<td>6.13</td>
<td>3.23</td>
<td>6.32</td>
<td>3.62</td>
</tr>
<tr>
<td>Baseline SWS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4h</td>
<td>48.19</td>
<td>12.41</td>
<td>40.69</td>
<td>21.42</td>
</tr>
<tr>
<td>5-8h</td>
<td>62.24</td>
<td>8.96</td>
<td>58.62</td>
<td>13.32</td>
</tr>
<tr>
<td>9-12h</td>
<td>54.46</td>
<td>13.53</td>
<td>47.44</td>
<td>15.30</td>
</tr>
<tr>
<td>13-16h</td>
<td>35.15</td>
<td>14.19</td>
<td>33.60</td>
<td>9.89</td>
</tr>
<tr>
<td>17-20h</td>
<td>51.47</td>
<td>6.63</td>
<td>42.39</td>
<td>10.06</td>
</tr>
<tr>
<td>21-24h</td>
<td>28.43</td>
<td>14.89</td>
<td>29.73</td>
<td>18.26</td>
</tr>
<tr>
<td>Total</td>
<td>46.66</td>
<td>11.77</td>
<td>42.08</td>
<td>14.71</td>
</tr>
<tr>
<td>Baseline Wake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4h</td>
<td>51.10</td>
<td>12.50</td>
<td>57.78</td>
<td>22.32</td>
</tr>
<tr>
<td>5-8h</td>
<td>28.59</td>
<td>11.80</td>
<td>34.44</td>
<td>13.84</td>
</tr>
<tr>
<td>9-12h</td>
<td>40.27</td>
<td>14.51</td>
<td>45.53</td>
<td>16.59</td>
</tr>
<tr>
<td>13-16h</td>
<td>55.51</td>
<td>16.74</td>
<td>57.02</td>
<td>12.92</td>
</tr>
<tr>
<td>17-20h</td>
<td>35.83</td>
<td>7.49</td>
<td>46.56</td>
<td>13.49</td>
</tr>
<tr>
<td>21-24h</td>
<td>60.51</td>
<td>22.22</td>
<td>53.48</td>
<td>25.55</td>
</tr>
<tr>
<td>Total</td>
<td>45.30</td>
<td>14.21</td>
<td>49.14</td>
<td>17.45</td>
</tr>
</tbody>
</table>

**Predictors of Shuttle Avoidance Performance**

The percent total duration of REM, SWS and wake did not predict shuttle avoidance performance in any of the baseline recording periods (Table 3).
Table 3. Correlation coefficients (r) and statistical significance (p) between percent correct avoidances at re-test and sleep variables (percent total recording time of REM, SWS and wake) in each of the 4-hour recording periods. No significant correlations were observed at p < 0.0083, corrected using the family-wise Bonferroni method for six comparisons evaluated at df = 35.

<table>
<thead>
<tr>
<th>Period</th>
<th>1-4h</th>
<th>5-8h</th>
<th>9-12h</th>
<th>13-16h</th>
<th>17-20h</th>
<th>21-24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>REM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.39</td>
<td>0.02</td>
<td>0.00</td>
<td>-0.02</td>
<td>0.16</td>
<td>-0.19</td>
</tr>
<tr>
<td>p</td>
<td>0.18</td>
<td>0.10</td>
<td>0.33</td>
<td>0.10</td>
<td>0.41</td>
<td>-0.08</td>
</tr>
<tr>
<td>SWS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.29</td>
<td>-0.12</td>
<td>-0.22</td>
<td>-0.18</td>
<td>-0.06</td>
<td>0.42</td>
</tr>
<tr>
<td>p</td>
<td>0.02</td>
<td>0.89</td>
<td>0.98</td>
<td>0.92</td>
<td>0.36</td>
<td>0.29</td>
</tr>
<tr>
<td>Wake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.30</td>
<td>0.53</td>
<td>0.05</td>
<td>0.56</td>
<td>0.02</td>
<td>0.63</td>
</tr>
<tr>
<td>p</td>
<td>0.10</td>
<td>0.49</td>
<td>0.22</td>
<td>0.30</td>
<td>0.76</td>
<td>0.01</td>
</tr>
</tbody>
</table>

The groups did not differ significantly in the number of sleep spindles/minute across baseline recording periods (Table 4).

Baseline spindle density for all rats significantly negatively correlated with shuttle avoidance performance only in the 21 to 24 hour period (Table 5 and Figure 18A; r(29) = -0.47, p = 0.007). One non-learning rat appeared to be a possible outlier, however, when removed from the analysis the correlation remained significant. Thus, this case was included. On training day 1, only change in spindle density in the 21 to 24 hour period significantly positively correlated with avoidances (Table 5 and Figure 18B; r(29) = 0.51, p = 0.003).

Table 4. Means (M) and standard deviations (SD) of spindle density (#/min) for each 4-hour period on baseline in learning (n=14) and non-learning rats (n=17).

<table>
<thead>
<tr>
<th>Period</th>
<th>Learning</th>
<th></th>
<th>Non-Learning</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>1-4h</td>
<td>8.14</td>
<td>4.12</td>
<td>9.39</td>
<td>3.91</td>
</tr>
<tr>
<td>5-8h</td>
<td>9.23</td>
<td>4.27</td>
<td>10.04</td>
<td>3.52</td>
</tr>
<tr>
<td>9-12h</td>
<td>9.14</td>
<td>3.35</td>
<td>10.34</td>
<td>3.42</td>
</tr>
<tr>
<td>13-16h</td>
<td>9.40</td>
<td>2.07</td>
<td>11.21</td>
<td>3.05</td>
</tr>
<tr>
<td>17-20h</td>
<td>9.77</td>
<td>2.12</td>
<td>11.51</td>
<td>3.54</td>
</tr>
<tr>
<td>21-24h</td>
<td>7.91</td>
<td>2.04</td>
<td>10.71</td>
<td>2.90</td>
</tr>
<tr>
<td>Total</td>
<td>8.93</td>
<td>3.00</td>
<td>10.53</td>
<td>3.39</td>
</tr>
</tbody>
</table>
Figure 18. A: Scatterplot of the correlation ($r = -0.47$, $p = 0.007$) between baseline spindle density (#/min) from 21-24 h on the baseline recording and the percent correct avoidances on the re-test. Rats above the learning criterion indicated by the filled circles (n=14), non-learning rats by open circles (n=17). B: Scatterplot of the correlation ($r = 0.51$, $p = 0.003$) between the change in spindle density from 21-24 h and the percent correct avoidances on the re-test. Rats above the learning criterion for the shuttle task indicated by filled circles (n=14), non-learning rats by open circles (n=17). Dashed verticle line indicates no change from baseline. Points may represent more than one individual rat.
Table 5. Correlation coefficients (r) and statistical significance (p) between percent correct avoidances at re-test and spindle density in each 4-hour recording period from the baseline and Training Day 1 recordings. * correlation is significant at p < 0.0083, corrected using family-wise Bonferroni method for six comparisons evaluated at df = 29.

<table>
<thead>
<tr>
<th>Period</th>
<th>Baseline</th>
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<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>1-4h</td>
<td>-0.17</td>
<td>0.355</td>
<td>-0.13</td>
<td>0.483</td>
</tr>
<tr>
<td>5-8h</td>
<td>-0.09</td>
<td>0.625</td>
<td>0.27</td>
<td>0.137</td>
</tr>
<tr>
<td>9-12h</td>
<td>-0.17</td>
<td>0.337</td>
<td>0.22</td>
<td>0.226</td>
</tr>
<tr>
<td>13-16h</td>
<td>-0.25</td>
<td>0.159</td>
<td>0.41</td>
<td>0.019</td>
</tr>
<tr>
<td>17-20h</td>
<td>-0.19</td>
<td>0.286</td>
<td>0.44</td>
<td>0.013</td>
</tr>
<tr>
<td>21-24h</td>
<td><strong>-0.47</strong></td>
<td><strong>0.007</strong>*</td>
<td><strong>0.52</strong></td>
<td><strong>0.002</strong>*</td>
</tr>
</tbody>
</table>

Discussion

As we previously showed, rats could be separated into learners and non-learners following training for 50 trials per day over 2 days (Fogel, Smith and Beninger, 2009). Surprisingly, it was found that sleep spindle density during SWS in the 21 to 24 h period during baseline was negatively correlated with avoidance performance at re-test. Within the sample, non-learning rats had elevated spindle density compared to learning rats. Conversely, and as predicted during the same time period but following training, change from baseline in sleep spindle density was positively correlated with performance improvements, whereby learning rats generally had a learning-dependent increase in sleep spindle density, whereas non-learning was associated with small increases or decreases from baseline in sleep spindle density on training day 1. Sleep spindles did not correlate significantly with avoidance performance at any other time period.

Our previous investigations revealed two distinct and non-overlapping learning-related changes in sleep architecture following the first day of learning: 1) increased REM sleep from 17 to 20 h followed by 2) increased sleep spindle density from 21 to 24 h post-training (Fogel, Smith and Beninger, 2009; in press). The results presented here provide further support for the second step in the 2-stage model discussed by Fogel, Smith and Beninger (2009), suggesting that sleep spindles during the 21 to 24 h period are related to learning. Post-learning increases in sleep
spindles may serve to strengthen neocortical memory traces, consistent with findings in humans (Gais et al., 2002; Fogel and Smith, 2001; 2006) and rats (Fogel, Smith and Beninger, 2009; in press; Molle et al., 2009). We also showed for the first time that baseline sleep spindles could predict whether rats would be able to learn to make avoidance responses in the shuttle task. Other individual differences in sleep architecture did not predict avoidance performance.

Despite two days of acclimatization, simply exposing rats to the novel recording cages and recording apparatus may have lead to changes in sleep architecture. Previous studies have shown that exposing a rat to a new environment can lead to changes in sleep architecture (for review see Smith, 1985; also see Smith and Wong, 1991). It is possible that exposure to the recording environment elicited a change in sleep spindles in non-learning rats, but not learning rats at baseline. Decreased homeostatic regulation, or conversely, an increased response to irrelevant or maladaptive stimuli in non-learning animals may explain why elevated sleep spindles might relate to poorer performance. The use of more extensive acclimatization periods in future studies may make it possible to evaluate this hypothesis.

SWS is thought to regulate synaptic homeostasis (Tononi and Cirelli, 2006). The synaptic homeostasis hypothesis makes several predictions: 1) wakefulness results in increased synaptic potentiation, 2) increased synaptic potentiation is related to increased slow wave activity, 3) slow wave activity is involved in synaptic downscaling and 4) synaptic downscaling is related to improved performance. Borbely and Achermann (1999) have described two processes involved in sleep regulation including process C (circadian) which describes how sleep pressure changes over the 24-hour cycle, and process S (sleep) which accumulates exponentially over the course of wakefulness, and decreases exponentially with the onset of sleep. Process C is responsible for restricting sleep to an adaptively advantageous time. Process S is reflected by high maximal levels of slow wave activity at sleep onset, which then decline as sleep progresses, and is thought
to be responsible for the predominance of slow wave sleep early in the sleep cycle. Encoding new information throughout wakefulness comes at a cost: energy expenditure, increased metabolic demands, new synaptic growth. Tononi et al. (2006) propose that these factors increasingly saturate our capacity to learn, and sleep serves to regulate synaptic strength and proliferation. The highly synchronized activity of slow wave sleep serves to regulate this activity. Slow wave activity has been proposed as one of the mechanisms involved in synaptic homeostasis (Tononi and Cirelli, 2006). Slow wave activity is temporally related to sleep spindles (Eschenko et al., 2009; Molle et al., 2002). Sleep spindles have been found to be related to memory consolidation processes (Clemens et al., 2005; 2006; Fogel et al., 2001; 2006; 2007; Gais et al., 2002; Meier-Koll et al., 1999) and may be involved in neocortical synaptic plasticity (Steriade and Timofeev, 2003; Rosanova and Ulrich, 2005). The role for sleep spindles in synaptic homeostasis remains to be investigated. As the data presented here suggest, learning-dependent increases in sleep spindles may reflect memory consolidation processes in response to new learning. If synaptic homeostasis (i.e., a return to a depotentiated state) is necessary for effective memory consolidation, what would be the result of unresponsive homeostatic regulation? A high number of baseline sleep spindles in non-learning rats may represent active consolidation to irrelevant or maladaptive information, and perhaps, poorly regulated homeostatic function. In other words, non-learning rats may be at or close to a ceiling effect in terms of the capacity for sleep spindles to benefit learning and memory. Conversely, while there was no significant group difference at baseline, the negative correlation between baseline sleep spindles and avoidance performance indicate that in learning rats, a lower number of spindles at baseline allow for an increase in spindles necessary for memory consolidation.

Extreme spindle activity has been observed in human studies of children with learning disabilities who are developmentally delayed (Gibbs and Gibbs, 1962; Shibagaki, Kiyono and
Watanabe, 1983). Bixler and Rhodes (1968) observed this pattern in mentally retarded adolescents along with a higher density of sleep spindles and suggested that this aberrant activity may result from a lack of normal inhibitory activity in thalamocortical networks. The results from the current investigation suggest that while sleep spindles may be involved in memory consolidation, in some cases, high levels of sleep spindles at baseline may be maladaptive. The focus of the relationship between baseline spindles and IQ in humans has been conducted using University students, not across a wide range of IQ scores. Considering the positive correlation observed in normal and high IQ individuals, and the high density of sleep spindles observed in developmentally delayed children, across a broader range of IQ scores the relationship between spindles and IQ may be curvilinear: high spindles in high IQ individuals, low spindles in moderate IQ individuals, but also high in very low IQ individuals. Studies using a wider range of IQ scores are needed to evaluate this hypothesis. In rats, this relationship appears to be linear, and the direction of the relationship driven by high spindle density in non-learning rats.

The results presented here are purely correlational and bivariate in nature. With continued research, more predictors of learning ability may provide a more complete model of the relationship between sleep and memory performance. Such a model could be used to assess how sleep mediates or moderates memory consolidation, and related homeostatic processes. Future research could employ lesion or drug techniques to use causal manipulations to investigate whether inducing similar conditions (i.e., decreased baseline spindles) would lead to improved memory performance. Similarly, drug manipulations (either central or peripheral) could be used during the post-training period where spindle increases predominate to assess whether interfering or promoting spindle activity affects memory performance.

Future studies could investigate whether baseline spindles predict memory performance in other tasks that are dependent on dissociable memory systems (i.e., spatial vs. non-spatial).
Learning dependent increases in the transition from SWS to REM, which is particularly rich in spindle activity has been observed following object recognition (Shiffelholz and Aldenhoff, 2002) and increased sleep spindles have been observed following odor-reward association task training (Eschenko et al., 2006; Molle et al., 2009). It is not known whether sleep spindle density predicts performance in these tasks.

In summary, rats in the non-learning condition appear to have a high density of sleep spindles at baseline, and this is unaffected by training. On the other hand, learning rats have a lower spindle density at baseline, but have a learning-related increase in sleep spindle density. Sleep spindle density correlates with learning potential in humans, and across studies, there is some evidence to suggest that this relationship may be curvilinear. In rats, baseline sleep spindle density predict whether a rat will be able to learn to make avoidance responses in the two-way shuttle avoidance task, however this relationship appears to be linear and negative. The present investigation suggests that sleep spindles are involved in sleep-dependent memory consolidation, but also may be involved in synaptic homeostasis.
Chapter 5
General Discussion

Chapter 1 was intended to provide a general introduction to the topics covered in greater detail in the manuscripts comprising chapters 2, 3 and 4. In chapter 2, a 2-stage model of sleep-dependent memory consolidation was presented based on the characteristics of the learning-related changes in sleep architecture. It was observed that following training on the two-way shuttle avoidance task for 50 trials/day over 2 days, an increase from baseline in the duration of rapid eye movement (REM) sleep occurred from 13 to 20 hours on the first post-training day compared to non-learning animals. During this period, an increase in theta activity was also observed. Following this, an increase from baseline in the number of sleep spindles/minute and sigma power during slow wave sleep (SWS) occurred from 21-24 hours in learning rats compared to non-learning animals. In chapter 3, the first step of the 2-stage model was tested to determine if interfering with the initiation of REM sleep would affect sleep-dependent memory consolidation. Direct injections of the gamma-aminobutyric acid (GABA)\(_A\) agonist baclofen into the region of the pedunculopontine (PPN) and deep mesencephalic reticular nuclei (DpMe) during the period when increased REM sleep was expected to be maximal in learning rats, resulted in reduced REM sleep and impaired subsequent performance. In Chapter 4, the relationship between baseline sleep spindles and shuttle performance was investigated. Baseline sleep spindles were negatively correlated with shuttle performance in the 21-24 hour period, the same time period when learning-dependent changes in sleep spindles were observed in chapter 2 and 3, and when sleep spindle density was positively correlated with shuttle performance. Taken together the results presented here suggest that both REM and SWS are necessary for the consolidation of avoidance learning.
Characterizing the REM sleep window

Post-learning increases in REM sleep that persist for several days have been previously observed (Smith and Lapp, 1986; Smith, Young and young, 1980; Smith and Kelly, 1988). Increased REM sleep over the 24-hour cycle occurs as a result of increased REM sleep in discrete (about 4-hour) periods termed REM sleep windows (RSW). Previous studies have shown that the timing of the RSW varies according to the intensity of the training regime (Smith and Lapp, 1986; Smith, Young and Young, 1980) and when trained for 50 trials/day over 2 days on an avoidance task, increased REM sleep occurs on the first post-training day, but not on the second post-training day. Following day 2, increased REM sleep persists for at least one week (Smith and Lapp, 1986; Smith, Young and Young, 1980). Increased REM sleep results from an increased number of REM episodes, rather than a lengthening of the duration of REM episodes (for review see Smith, 1985; but see Smith, Young and Young, 1980). The results reported in Chapter 2 are consistent with these findings, and further characterize the nature of the changes of REM sleep following learning. Increased EEG power in the theta and beta bands during REM sleep inside the RSW were identified, and suggest that theta activity is an electrophysiological marker and may contribute to memory consolidation processes specific to REM sleep.

Theta activity is normally the dominant frequency observed in the EEG of REM sleep. Theta activity is thought to be one of the frequencies by which the hippocampus and the neocortex communicate (Klimesch, 1999). REM sleep may provide ideal conditions for the replay of previously learned information and hippocampo-neocortical communication. One of the distinguishing features of REM sleep is the bursts of rapid eye movements observed during phasic REM sleep. In humans, REM sleep parameters have been found to increase following declarative learning in humans (Fogel, Smith and Cote, 2007; Smith and Lapp, 1991). Rapid eye movements coincide with ponto-geniculo-occipital (PGO) activity, and in the rat, the pontine
component (p-wave) has been found to increase following avoidance learning in rats (Datta, 2000; Mavanji and Datta, 2003). Theta rhythms are another characteristic feature of REM sleep (Bland and Oddie, 1998; Jouvet et al., 1965). Cortical theta is thought to be involved in long-term potentiation (LTP) (Pavlides et al., 1988) and increased following declarative learning in humans during REM sleep (Fogel, Smith and Cote, 2007). In Chapter 2, EEG was measured from epidural electrodes, thus the exact source of theta activity cannot be determined from these data. However, electrodes were placed overlying the hippocampus, and one of the major sources of theta recorded in this way originates from the hippocampus (Winson, 1974). Theta rhythms in humans are measured from scalp derivations, and several theta generators (both cortical and hippocampal) contribute to EEG theta (Kahana, Seelig and Madsen, 2001). While learning-related increases in theta reported in Chapter 2 are similar to findings reported in humans (Fogel, Smith and Cote, 2007), it cannot be determined whether the source of these rhythms originates from the hippocampus. Karashima et al. (2004) demonstrated that theta activity is correlated with both p-wave activity and REM density. The PPN is one structure that could act as a common generator for both phasic activity during REM sleep (Koyama and Sakai, 2000; Saponjic, Radulovacki and Carley, 2003) and hippocampal theta during REM sleep (Datta and Patterson, 2003; Kirk, 1998). Thus, the results of infusions of the GABAergic receptor agonist baclofen into the PPN region reported in Chapter 3 suggest that inhibition of the PPN region may disrupt the generation of theta or p-wave activity, and support previous literature identifying the presence of the RSW. This hypothesis remains to be directly investigated. These findings are consistent with studies in humans, and further characterize the learning-related changes of the EEG during this period, suggesting that theta activity is involved in sleep-dependent memory consolidation processes.
Sleep deprivation studies have also demonstrated that the RSW is a discrete period when memory consolidation may take place. Selective REM sleep deprivation has been used to characterize the timing of the RSW. Sleep deprivation for four hours coincident with the RSW time period impaired performance in rats trained for 50 trials/day over 2 consecutive days on the 2-way shuttle avoidance task (Smith and Lapp, 1986). On the other hand, sleep deprivation for four hours at times outside the RSW did not impair performance (Smith and Butler, 1982). When REM sleep was allowed during the RSW, but not during any other time, performance did not significantly differ from controls (Smith and Butler, 1982; Leagault, Smith and Beninger, 2004; 2006). In Chapter 3, the involvement of the PPN for sleep-dependent memory consolidation was investigated. When the GABA\textsubscript{B} agonist baclofen was infused into the PPN at a time coincident with RSW onset, REM sleep was reduced for a period of about 4 hours, and subsequent memory performance was impaired. The PPN has been implicated in the generation and maintenance of REM sleep (Rye, 1997; Sapin et al., 2009; Steriade and McCarley 1990) and incentive learning (Satorra-Marín et al., 2001). The present study suggests that decreased GABAergic transmission in the PPN region reduces REM sleep, impairing subsequent performance. These results suggest that normal PPN functioning is required during periods where memory consolidation occurs.

Neurodegeneration of cells in the region of the PPN is observed in the early stages of Parkinson’s disease (Braak et al., 2003). The results presented here suggest that cognitive deficits observed in Parkinson’s disease (Peretta, Pari and Beninger, 2005) may result from the impact of REM sleep disruption on memory consolidation.

The results across all three studies reported here suggest that there may be a SWS window, marked by increased sleep spindles following the RSW. In Chapter 2, learning-dependent increases in sleep spindles were observed in rats that learned the task, but not in non-learning rats. Sleep spindles have been found to increase in a learning-dependent manner in rats
(Eschenko et al., 2006) and in humans (Gais et al., 2002; Fogel and Smith, 2001; 2006). In Chapter 3, when the learning-dependent changes in REM sleep were reduced by GABAergic blockade of REM-on cells in the PPN/DpMe region of the brainstem, the subsequent increase in sleep spindles was not observed, suggesting that increased sleep spindle occurrence is sequential being dependent on increases in REM sleep to further consolidate the neocortical memory trace. The results presented in Chapter 4 provide additional support for the existence of a SWS window. Correlations with shuttle performance were significant only during the 21-24 hour period, both at baseline and with post-training changes on training day 1. The correlation with baseline spindles was negative suggesting that non-learning rats may be close to a spindle density ceiling, leaving little or no capacity for a learning-related increase in spindles following learning. On the other hand, learning rats had fewer spindles at baseline, and had a learning-related increase following training. This increase in spindles also occurred in the same time period (0700-1100 h) 21-24 h post-training, when the correlation with the difference in spindle density from baseline to post-training sleep was significantly positive. Learning-related changes in sleep including increased REM, increased EEG spectral power (e.g. theta during REM, sigma during SWS) and sleep spindles were only observed on the 1\textsuperscript{st} training day, but not on the 2\textsuperscript{nd} training day despite that in learning rats, avoidance performance continued to improve thereafter. These results are consistent with a previous study using the same training regime (Smith and Lapp, 1986) whereby increased REM sleep was observed on the 1\textsuperscript{st} training day, but not on the 2\textsuperscript{nd}. REM increases were again observed from the 3\textsuperscript{rd} to the 7\textsuperscript{th} post-training day, suggesting that memory consolidation processes continue for at least one week after training.

\textit{2-stage models of sleep and memory}

Several models have been described implicating both non-REM and REM sleep in different aspects of memory consolidation. One of the earliest models was the sequential
hypothesis described by Giuditta (1985; 2003). They observed that certain sequences of sleep
state transitions (e.g., SWS → transition sleep (TS) → REM) predominated post-learning sleep in
fast-learning rats as compared to slow learning or non-learning rats. Furthermore, these sequences
also appear imbedded in longer trains of sequences (e.g., wake (W) → SWS → TS → REM →
SWS → TS → W…) that differ between learners and non-learners. For example, rats that had
sequences containing TS → W were more frequent among fast learning rats (Piscopo et al.,
2001). It has not been directly investigated how these sequences and trains of sequences
correspond to overall changes in sleep architecture (e.g., increased percent REM sleep duration in
learning rats). Certain sleep states predominate particular sequences, thus an increase in certain
sequences could result in changes in sleep architecture.

The results presented in Chapter 2 suggest that trains of sequences rich in REM sleep
would be postulated to predominate during the 17-20 h window, and that trains of sequences rich
in SWS and TS sequences (periods of high spindle activity) may predominate from 21-24 h. Our
results are consistent with evidence supporting the sequential hypothesis whereby REM
containing sequences (SWS → TS → REM) and SWS containing sequences (SWS → TS → W)
were more frequent in learning rats and were correlated with performance on the shuttle
avoidance task (Giuditta et al., 2003). Whether or not these trains of sequences drive changes in
post-learning sleep architecture remain to be tested, and would bridge the levels of analysis used
by Giuditta et al., the current investigation and others (for review see Hennevin and Leconte,
1971; Smith, 1985). The current data are consistent with the main premise which supports the
sequential hypothesis, that both SWS and REM sleep are sequentially involved in sleep-
dependent memory consolidation.

While most other investigations have focused on the role of REM sleep in memory
consolidation, others such as Buzsaki et al. (1996) have suggested that several features of SWS
are involved in memory consolidation and are excellent candidates for markers of synaptic plasticity. During wakefulness, theta grouped by high frequency activity bursts in the beta/gamma range is involved in the encoding process to form a hippocampal-neocortical memory trace. During SWS, sharp waves originate in bursts of excitatory activity in the CA3 region of the hippocampus, lasting 40-100 ms, and are associated with fast ripple activity (~200 Hz) originating from the CA1 pyramidal layer of the hippocampus (Buzsaki, 1996). Buzsaki has suggested that bursts of sharp wave activity are ideal for the induction of LTP for several reasons: 1) the burst duration of sharp wave activity is consistent with the time required for the N-methyl-D-aspartic acid (NMDA) receptor to allow Ca^{2+} influx (Kandel, 1981), 2) the frequency range ~200 Hz that the network of hippocampal neurons outputs is the ideal frequency for inducing LTP (Douglas and Goddard, 1975), 3) synaptic potentiation is more likely with an increase in network excitation (Gustafsson and Wigstrom, 1988), and 4) sharp waves are a means for extra-hippocampal communication (Chrobak and Buzsáki, 1994; Siapas and Wilson, 1998) to neocortical structures. Temporal correlations between sleep spindles and ripple activity have been observed (Siapas and Wilson, 1998; Molle et al., 2009), suggesting that these two features of SWS are functionally linked.

Our results show that following avoidance learning, increased sleep spindles are observed, which may reflect the cortical aspect of hippocampo-neocortical communication. Moreover, our results go beyond Buzsáki’s model suggesting that features of SWS such as the sleep spindle are involved in memory consolidation, but represent further neocortical refinement following reactivation that may occur during REM sleep. Thus, both non-REM and REM sleep may be necessary for efficient memory consolidation to occur. During SWS, hippocampal synaptic plasticity is suppressed (Bramham and Srebro, 1989; Leonard, McNaughton and Barnes, 1987) but returns during REM sleep when theta rhythms are present (Ranck, 1973). Theta
rhythms are one of the frequencies that the hippocampus uses to communicate with other structures (Larson and Lynch, 1986; Larson Wong and Lynch, 1986). Thus, REM sleep would seem to have the ideal conditions for hippocampo-neocortical transfer of information, and SWS may be responsible for further neocortical strengthening via increased sleep spindle activity (when further intra-hippocampal strengthening would not occur), thus enabling the neocortical trace to become increasingly independent of the hippocampus. The novelty-familiarity of a remembered location modulates theta frequencies (Poe et al., 2000). When exposed to a novel environment, place cells fire preferentially at theta peaks (thought to depolarize cells, leading to further potentiation), whereas when rats are exposed to a familiar environment, place cells fire preferentially at theta troughs (thought to hypopolarize cells, leading to depotentiation). The phase reversal from REM theta peak (novel) to trough (familiar) takes place over several days (Poe et al., 2000). On training day 1, cell firing was coincident with theta peaks. On each succeeding day, timing of the cell firing deviated further from the theta peak and by day 5, when the task was mastered, cell firing was coincident with theta troughs. Theta phase remained unchanged during wakefulness over the same number of days.

While there is much consensus in the human literature concerning the involvement of stage 2 sleep in the consolidation of cognitively simple motor learning, and the role of REM sleep in providing insight for novel and cognitively complex tasks, dissociable changes specific to a particular sleep state following declarative learning seems to involve Stage 2, SWS and REM sleep. One explanation for the various findings in the literature is that hippocampal-dependent learning requires sequential processing during sleep to be consolidated into a more enduring form. This is not surprising considering the fact that the hippocampus has a time-limited role in hippocampal-dependent memory. Thus at least two steps would be required for the consolidation of declarative memories: 1) hippocampal-neocortical dialogue and 2) neocortical
consolidation/refinement. Theta rhythms are considered to be one of the frequencies that the hippocampus communicates with other structures (Klimesch, 1999) and have been implicated in the induction of LTP (Larson and Lynch, 1986; Trepel and Racine, 1998). Theta power has been found to increase in humans during REM sleep following paired associates learning (Fogel, Smith and Cote, 2007), and the results reported in Chapter 2 also identified theta increases in learning rats compared to non-learning rats during the RSW. When REM sleep in the RSW is reduced as demonstrated in Chapter 3, rats are unable to learn the avoidance task. Increased theta activity may represent reactivation indicated by increased hippocampal-neocortical dialogue, and may even be involved in the induction of LTP in cortical areas to form or strengthen neocortical memory traces. For the reactivated memory trace to become increasingly independent of the hippocampus, cortico-cortical connections would also require strengthening. Sleep spindles have been implicated in cortical synaptic strengthening (Steriade and Amzica, 1998; Steriade, 1999; Destexhe and Sejnowski, 2001), have been shown to induce cortical LTP (Rosanova and Ulrich, 2005) and has been observed to increase following learning in rats (Eschenko et al., 2006; Molle, 2009) and humans (Gais et al., 2002; Fogel and Smith, 2001; 2006). Thus, sleep spindles may serve as a marker or potential mechanism for neocortical memory consolidation.

**Relation with memory theory**

*Multiple trace theory*. Multiple trace theory stipulates that both the neocortex and the hippocampus are involved in the initial encoding, consolidation and retrieval processes for episodic memory. Damage to the hippocampus results in anterograde amnesia and retrograde amnesia that can extend back for decades (Haist et al., 2001) with a flat gradient over time (i.e., both recent and remote memory impairment) for episodic memory with complete hippocampal lesions. The extent of the lesion to the hippocampus is related to the severity of amnesia, with more limited damage sparing more recent episodic memories. On the other hand, the
Hippocampus has a time-limited role in the consolidation of semantic information (Nadel and Moscovitch, 1997). Damage to the hippocampus results in temporally-graded retrograde amnesia (recent but not remote memory impairment). Consolidation occurs as a result of reactivation of the memory trace, and over time, transforms the trace into a stronger and more highly interconnected network of memory traces.

The question arises: how do the data presented here fit with existing models of memory consolidation? While the two-way active avoidance task is not purely a hippocampal task, hippocampal lesions do affect performance on this task (Olton and Isaacson, 1968). Furthermore, prolonged theta activity has been observed immediately (several minutes) following 2-way shuttle avoidance training (Bramham, Maho, Laroche, 1994), suggesting that 1) the hippocampus mediates task acquisition, and 2) theta activity might reflect hippocampo-neocortical dialogue for the short-term consolidation of the memory traces. The results presented in Chapter 2 suggest that REM sleep may be an ideal time for reactivation of the memory trace to occur, and that a similar type of brain activity (i.e., theta activity) is responsible for this reactivation. Since the 2-way shuttle avoidance task is not purely hippocampal-dependent, extra-hippocampal consolidation mechanisms may also be involved.

The data presented here suggest that there may be a second step involved in the strengthening of neocortical traces, which may still involve the hippocampus. Hippocampal ripples are temporally synchronized with sleep spindle activity (Molle et al., 2009). Sleep spindles have been suggested as an ideal mechanism for neocortical consolidation, and have been found to induce neocortical LTP (Rosanova and Ulrich, 2005; Werk, Habour and Chapman, 2005). In Chapters 2 and 3, a learning-dependent increase in sleep spindles was observed following increases in REM sleep. This sequence of events fits well with the basic tenets of Multiple Trace Theory, in that reactivation involves the creation of new hippocampo-neocortical
connections. Proliferation of neocortical interconnections would require a mechanism for neocortical LTP, a process that might be subserved by increased sleep spindle activity. Support for this type of dissociation also comes from human studies where increased sleep spindles were observed following motor procedural learning, but not declarative learning (Fogel, Smith and Cote, 2007). On the other hand, increased theta and sigma activity during REM sleep was observed following declarative learning.

**Motor Skill Learning.** Much of the evidence to either support or refute Multiple Trace Theory comes from the study of the role of the hippocampus (and other structures of the medial temporal lobe). Not all types of memory are so intricately linked to hippocampal function as semantic or episodic memory, thus the Multiple Trace Theory is not a complete account of consolidation processes for all types of memory. Motor skills learning is one such example where the hippocampus has a more limited role. Doyon and Ungerlieder (2002) have outlined a model that describes the neural substrates of two kinds of motor skills: motor sequence learning and motor adaptation. These types of tasks are typically learned by physical practice, and as practice progresses, the movements become increasingly automated: more fluid and effortless. Two distinct phases can be observed with practice: first, a rapid improvement in performance over the course of a single (or series) of practice sessions, followed by a more gradual improvement characterized by changes that occur, over the course of days or even weeks (Nudo et al., 1996; Karni et al., 1998).

The largest performance gains without additional practice were observed after an intervening period of sleep (Doyon et al., 2009; Walker et al., 2002), suggesting that sleep might serve to further consolidate motor sequence learning. Two cortical-subcortical loops have been identified in motor skills learning. A cortico-cerebello-thalamo-cortical loop and a cortico-striato-thalamo-cortical loop (Middleton and Strick, 1997). The former is involved in motor adaptation.
where early and rapid performance improvements are observed, and the later is involved in more gradual performance improvements that takes place over days (Doyon and Ungerlieder, 2002). Support for this model has come primarily from neuroimaging studies whereby activation in the striatum, cerebellum, motor cortex, supplementary motor area, prefrontal, parietal and limbic areas occurs during the early stages of both motor sequence learning and motor adaptation. Once performance has reached asymptote, and consolidation has taken place, for motor adaptation, the cerebellum and associated cerebral areas are active during task execution. Conversely, for motor sequence learning, the cerebellum is no longer required, and activation is observed in the striatum and associated motor cortical regions.

Sleep is thought to play a role in the consolidation of motor sequence learning (Walker et al., 2002) independently of circadian phase (Fischer et al., 2002). On the other hand, consolidation of motor adaptation learning does not appear to be sleep-dependent (Doyon et al., 2009). Using positron emission tomography (PET) imaging, Maquet et al. (2000) investigated whether brain activity during REM sleep changed as a result of previous waking experience on a sensorimotor task. It was found that brain areas that were active during training were reactivated during REM sleep compared to untrained subjects including the cuneus, left premotor cortex and mesencephalon. Thus, reactivation of structures involved in the acquisition of sensorimotor learning is observed during REM sleep. This reactivation is thought to be involved in consolidation processes. Sleep spindles have been found to increase following motor learning in humans (Fogel and Smith, 2006; Fogel, Smith and Cote, 2007). The results presented in Chapters 2 and 3 suggest that for avoidance learning, which may also depend on neocortical areas, sleep spindles may similarly be involved in memory consolidation processes.

**Incentive learning.** How is an incentive stimulus paired with an unconditioned stimulus from the environment and how does this pairing result in increased behavioural responding?
Bolles (1970) has proposed that avoidance responses can only be elicited quickly and reliably if
1) they are part of an animal’s repertoire of species specific defense reactions, such as running, freezing, rearing or threaten, and 2) avoidance responses (i.e., running) can only be elicited if competing species specific defense responses (i.e., freezing) are suppressed. Under these circumstances, other responses cannot be learned at all, or only after extensive training. Two-way shuttle avoidance learning poses an intriguing challenge to explain within the framework of classical or operant conditioning since not all rats learn to avoid the aversive stimulus. By comparison, one-way shuttle avoidance learning can be easily learned in few trials. One resolution to this issue is that avoidance responses in the two-way shuttle do not result in the rat fleeing to a location that is invariably “safe” (i.e., without an aversive stimulus), rather, they must return to a location where they once received shock. In other words, two-way shuttle avoidance learning can be considered to be more difficult to learn than one-way shuttle avoidance learning since an avoidance response is less effective to achieve the desired result (removal from the aversive situation), and competing species specific defense reactions may not be suppressed as readily.

A model for investigating the mechanisms involved in the establishment of incentive learning has been proposed by Beninger (1983; Beninger and Miller, 1998). The basal ganglia are structured in a way that they receive environmental input from sensory information via afferent projections from cortical sensory areas to the striatum and output efferent projections to motor nuclei (Imperato et al., 1981). Likewise, dopamine neurons that also synapse in the striatum receive afferent sensory information from the entorhinal and pyriform cortex, allocentric information from the hippocampus (Heimer and Wilson, 1975) and dopamine neurons in the substantia nigra have efferent output to the motor areas such as the striatum (Nauta et al., 1978). The glutamateergic sensory cortical afferents and dopaminergic reward-signal afferents synapse on
the same dendritic spine of the efferent motor output neuronal population (Freund et al., 1984; Kubota et al., 1987). These afferents also synapse on cholinergic interneurons in the striatum that, in turn, synapse on output neurons. Thus, the striatum may serve as a relay between sensory input and motor output where information about unconditioned environmental stimuli and information about behaviourally relevant stimuli converge and project to motor output areas. Cortical afferents synapse at cholinergic interneurons in the striatum. These interneurons may serve as a muscarinic interface between cortical sensory input and motor output. Beninger (Beninger, 1983; Beninger and Miller, 1998) proposed that dopamine afferents to the striatum also synapse at motor output nuclei. In summary, when a rat explores his environment, a variety of sensory information reaches the striatum, activating the glutamate receptors, coupling adenylate cyclase to nearby dopamine receptors. If the rat encounters a stimulus that is rewarding paired with an unconditioned stimulus from the environment, a reinforcing dopamine signal may strengthen the connection at the glutamatergic synapses since the dopamine signal was enhanced by the adenylate cyclase bound to the post-synaptic dopamine receptors. In turn, this leads to the formation of cyclic adenosine monophosphate to increase the function of the glutamate receptors, potentiating the cortical afferents to generate the motor response that resulted in the encounter with the reinforcing stimulus. Legault, Smith and Beninger (2006) have demonstrated that intra-striatal infusions of either acetylcholine or dopamine receptor antagonists during a known RSW for the radial arm maze impair subsequent learning. Infusions outside the established window (Legault, Smith and Beninger, 2004) did not impair subsequent performance. These results suggest that intact cholinergic and dopaminergic transmission in the striatum is necessary for memory consolidation for tasks involving incentive learning at times when REM sleep has been found to be necessary for normal memory performance. The present studies do not test this hypothesis directly, but are consistent with literature suggesting that acetylcholine and dopamine
are involved in sleep dependent memory consolidation during REM sleep, and more generally, that REM sleep is involved in the consolidation of incentive learning.

**Limitations**

The current series of studies have several limitations. Most notably, for all three studies, while post-hoc assignment to learning condition may afford a comparison between rats who learned and those who did not under the exact same training conditions, rats could not be randomly assigned to group. Thus, pre-existing characteristics could not be randomized across conditions, thereby making any conclusions from these data correlative in nature.

In addition, in Chapter 3, drug infusions were only made during the period previously identified as having increased REM (i.e., the RSW). Infusions outside these time frames would provide more conclusive evidence that REM only during the RSW was involved in memory consolidation, and not activity outside these periods. Extensive work by Smith (for review see 1985; 1995; 1996; 2003) has shown that memory performance is impaired when REM sleep deprivation occurs during periods of increased REM. When REM sleep (and SWS) is permitted during the RSW, but not at any other time across the circadian cycle, performance in REM deprived rats does not significantly differ from normally rested controls. Furthermore, LeGault, Smith and Beninger (2004) injected rats with a cholinergic receptor antagonist during a known RSW which impaired performance on the radial arm maze, but not when injected outside the RSW. Thus, it would be expected similarly, that infusions of baclofen into the region of the PPN/DpMe outside the RSW would reduce REM sleep during that time but not impair subsequent performance.

The timing of the RSW varies according to a number of factors including the training regime (Smith and Lapp. 1986; Smith, Young and Young, 1980) and the type of task (Legault,
Smith and Beninger, 2004, 2006; Smith and Conway, 1998; Smith, Conway and Rose, 1993; Smith and Rose, 1992). The timing of the RSW in the present studies differed from a previous study using the same task and training regime (Smith and Lapp, 1986) but a different light-dark schedule. Thus, the time of testing relative to circadian phase may also affect the timing of the RSW. Further research is required to test this hypothesis.

**Future Directions**

At present, the results from Chapters 3 and 4 provide only a partial test of the model presented in Chapter 2. Additional research is needed to provide a more complete test of the hypotheses of the 2-stage model. Previous studies have found that the timing of the RSW varied in a dose-dependent manner depending on the training regime. One of the underlying assumptions made in the 2-stage model is that learning-dependent changes in REM sleep precede changes in SWS. Support for this assumption could simply be provided by manipulating the training regime, for example by training rats for 100 trials in a single session, when the RSW has been previously identified at 1 – 4 hours post-training to determine if increased sleep spindles and sigma power followed in the subsequent 4-hours (from 5 – 8 hours). Furthermore, different methods of sleep deprivation could be used to selectively deprive rats of REM sleep alone, SWS alone, SWS and REM sleep, or total sleep deprivation. In a remarkable series of studies, Rechtschaffen et al. (1989) investigated the effects of prolonged total or selective sleep deprivation on a variety of physiological and behavioural indicators of homeostasis. The sleep deprivation and recording apparatus consisted of a cylindrical cage bisected by a barrier to create two separate housing compartments, each for one rat. The EEG of each rat was continuously recorded, and the EEG characteristics of the experimentally sleep deprived rat was used to control a motor that was used to make the floor of the cage rotate. The rat in the opposite compartment was used as a yoked control. When the floor rotated, the rats were obligated to walk to avoid being pushed into the
barrier. The motor could be activated by the presence of SWS and REM, REM alone or SWS alone. While the yoked control rats would have some sleep disruption, it would be administered randomly relative to their own sleep cycle. A similar sleep deprivation protocol could be used to investigate the relative contribution of REM and SWS at times inside and outside the RSW. Using a fully-crossed design, the effects of selective sleep deprivation for both the 100 trials per day in a single day and 50 trials per day over 2 days could be studied in rats at both sleep intervals (1 – 4 hours and 17 – 20 hours) in all sleep deprivation conditions, (SWS and REM sleep deprivation, SWS deprivation, REM deprivation and fully rested controls).

Polysomnographic recordings could be made throughout the post-training period allowing for a direct comparison between fully rested learning and non-learning animals, and sleep deprived groups (deprived at either 1 – 4 or 17 – 20 hours).

One of the major unanswered questions from the series of experiments is if a reduction of sleep spindles during the period following the RSW impairs subsequent memory performance. Conversely, it may also be possible to enhance sleep spindles during this period. A more comprehensive study of the effect of manipulating sleep spindles during the post-training period could provide a more complete test of the hypothesis that sleep spindles are causally involved in memory consolidation. By making infusions of either baclofen or CGP 35 348 into the various subregions of the thalamus (e.g., reticular nucleus, anterior and posterior medial nuclei) at different periods (as suggested above, following the 1-4 h RSW and 17-20 h RSW: from 5-8 and 21-24 hours) for the different training regimes (100 trials in one day, and 50 trials/day over 2 days) to assess whether enhancing or suppressing sleep spindle activity during critical periods may affect subsequent memory performance. A similar approach could also be used in the PPN region to ensure that infusions of either baclofen or CGP 35 348 do not affect memory performance at times outside the RSW. A preliminary study was conducted to assess the effects
of CGP 35 348 infusions into the reticular nucleus of the thalamus. It was hypothesized that sleep spindles would be reduced, and that subsequent performance would be impaired in drug-infused rats. Surprisingly, no change in sleep spindles was observed, and performance was unaffected. A brief summary of the methods and results are presented in Appendix A.

**Implications**

One of the earliest warning signs of Parkinson’s disease is the development of REM behaviour disorder (RBD). About 40% of cases diagnosed with RBD eventually develop Parkinson’s disease (Iranzo et al., 2006; Schenck et al., 1996), and about half of cases with Parkinson’s disease have RBD (Olson, Boeve and Silber, 2004). Cell death in the PPN occurs very early on in the brains of Parkinson’s patients (Braak et al., 2003) and, as the disease progresses, in other structures such as the basal ganglia. Memory deficits are also observed in Parkinson’s disease (Perretta, Pari and Beninger, 2005). The results presented in Chapter 3 suggest that disruption of REM sleep from increased inhibition of REM-on cells in the PPN result in reduced REM sleep and impaired memory performance. It is possible that either reduced REM sleep or damage to the PPN may be responsible for cognitive deficits observed in Parkinson’s disease. The results presented in Chapter 3 are consistent with the hypothesis that normal GABAergic transmission in the PPN is required for REM sleep and memory consolidation to take place, and dysfunction or neurodegeneration of the PPN region may contribute to sleep and memory deficits observed in Parkinson’s disease.

Damage to the thalamus is observed in Korsakoff’s Syndrome (Victor, Adams and Collins, 1971) and following stroke, results in selective memory deficits, affecting short-term memory (Winocur et al., 1984). This is in contrast to individuals with damage to the medial temporal lobe, with intact short-term memory, but display anterograde amnesia and temporarily-
graded retrograde amnesia (Wickelgren, 1968; Winocur, 1985). Thus, the thalamus appears to be involved in the acquisition or encoding of newly learned information. Several studies have demonstrated learning-dependent changes in sleep spindles in humans (Fogel and Smith, 2006; Gais et al., 2002; Morin et al., 2008) and rats (Eschenko et al., 2006). Evidence from neuroimaging studies has revealed not only increased activation of particular brain regions following learning, but moreover, reactivation of the same structures involved in encoding processes (Peigneux et al., 2003). A recent study where EEG and functional magnetic resonance imaging (fMRI) were coregistered has shown that among other structures, the thalamus is active during spindle activity (Schabus et al., 2007). The hemodynamic correlates of sleep spindle activity following learning remain to be investigated.
Summary and Conclusions

In conclusion, not all rats learn to make avoidance responses in the 2-way shuttle task. Learning rats exhibit increased REM sleep from 17-20 hours, followed by increased sleep spindles from 21-24 hours. Baseline sleep spindle density during the 21-24 hour period predicts whether rats will learn to make avoidance responses. Learning rats have less spindles at baseline than non-learning rats, but have a learning-dependent increase in spindles following training, whereas non-learning rats have no significant change in spindles. Increased GABAergic activity in the PPN/DpMe during the RSW reduces REM sleep, and impairs subsequent performance. The subsequent learning-dependent increase in sleep spindles is not observed in drug-infused rats. Thus, the present series of studies suggests that both REM and SWS are involved in sleep-dependent memory consolidation. REM sleep increases occur first, marked by increased theta power, which may reflect increased hippocampal-neocortical dialogue. This is followed by increased spindle density during SWS that may reflect increased neocortical consolidation.
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Appendix A

Supplemental Methods

Housing, behavioural testing and electroencephalogram (EEG) recording and analysis techniques were identical to those described by Fogel, Smith and Beninger, (2009; in press) and are presented in abbreviated form here. Thirty male Sprague-Dawley rats weighing 250-300 g were implanted with four EEG and two electromyogram (EMG) electrodes. Guide cannulae were implanted bilaterally into the reticular thalamus according to coordinates obtained from Paxinos and Watson (1998): from bregma posterior - 2.5 mm, lateral +/- 3.8 mm from the midline and 5.0 mm ventral from the surface of the skull. The cannulae were anchored to the skull with jeweller’s screws and dental acrylic. Stylets were inserted into the cannulae until injections were made.

After 14 days of recovery, 3 days of acclimatization, and 24 hours of baseline recording, animals were trained on the two-way avoidance task for 100 trials (50 trials/day) over two days and re-tested for 25 trials on day 3 between 1000 h and 1100 h. Rats were re-tested again for 25-trials one week later. Following the behavioural training session (training day 1), infusions of the GABA\textsubscript{B} receptor antagonist CGP 35 348 (1.5 nmol; 0.375 \(\mu\)g per side) were made via the implanted cannulae at 0700 h post-training over 30 seconds in a volume of 0.5 \(\mu\)L of phosphate buffered 0.9% saline per side using an injector attached by tubing to a microsyringe mounted on an infusion pump. The remaining rats received an infusion of saline (0.5 \(\mu\)L per side) into the reticular thalamus at the same time as the GABA\textsubscript{B} group. EEG was recorded for approximately 23 hours after training on both training day 1 and 2 beginning between 1000 and 1100 h. Rats in the learning group (n = 9) avoided footshock on 60% of the last 20 test trails on the re-test one day after training day 2. The remaining rats (n = 11) were assigned to the non-learning group. Half of the rats (n = 5) in the GABA\textsubscript{B} group (n = 10) performed above criterion.
**Supplemental Results**

**Behavioural Data**

Rats in the saline condition were clearly distinguished as learning or non-learning at re-test 1 day following training according to previously established criteria (Figure 19). An equal number of GABA\textsubscript{B} antagonist-infused rats performed above or below the 60% criterion. Learning rats increased avoidance responses over the training and testing sessions, whereas the non-learning rats did not, and the GABA\textsubscript{B} group performed at an intermediate level (Figure 19). A 3 x 4 (group x day) ANOVA for avoidance responses revealed a significant group by day interaction (F(6, 81) = 5.34, p < 0.0001). Avoidance responses significantly increased over the training and testing days as revealed by follow-up simple effects repeated measures ANOVAs for the learning group (F(3, 24) = 20.67, p < 0.0001) the GABA\textsubscript{B} group (F(3, 27) = 4.55, p = 0.01) but no significant change was observed in the non-learning condition (F(3, 30) = 1.48, p = 0.26).

**Figure 19.** Percent correct avoidances over training day 1, training day 2, 1-day re-test and 1-week re-test. Learning rats significantly increased avoidances (* p = 0.0001) over the training and re-test days, whereby all learning rats performed above 60% correct from the 1-day re-test onwards. There was no significant change in performance for non-learning rats over the course of training or at either re-test. The GABA\textsubscript{B} group improved performance significantly (+ p = 0.01), but at a slower rate than the learning rats.
Sleep Architecture Data

Learning rats had increased REM sleep during the fourth and sixth four-hour post-training periods on training day 1 as compared to the non-learning and GABA$_B$ groups (Figure 20). A 3 x 6 (group x period) ANOVA revealed a significant group by period interaction on training day 1 ($F(10,80) = 2.63$, $p = 0.012$) but not on training day 2 ($F(10,75) = 1.29$, $p = 0.25$) although REM sleep does appear elevated in the GABA$_B$ group during the third post-training period on training day 2. Follow-up tests of simple effects identified group differences in the fourth ($F(2,16) = 4.28$, $p = 0.033$) and sixth ($F(2,16) = 4.27$, $p = 0.033$) post-training period on training day 1.

To determine which groups differed from one another, follow-up t-tests revealed that the learning group had significantly higher REM sleep in the fourth post-training period than the non-learning ($t(10) = 2.33$, $p = 0.042$) and the GABA$_B$ group ($t(12) = 2.50$, $p = 0.028$), and significantly higher REM during the sixth post-training period than the GABA$_B$ group ($t(12) = 3.33$, $p = 0.006$). The RSW appeared earlier than that reported in Chapter 3, but overlapping with the RSW reported in Chapter 2. This could be due to sampling error or interindividucal differences in the timing of REM increases and performance improvements.

There were no group differences between the non-learning and GABA$_B$ group at either post-training period. ANOVA revealed that there was no significant group by period interaction for percent duration of SWS or wake on training day 1, or on training day 2 (data not shown).
Figure 20. Percent of total recording time for each post-training 4-hour period on training day 1 and 2 for REM sleep. Increased REM sleep was observed in the learning group in the 4th and 6th post-training period on training day 1 (* p < 0.05). The 4-hour periods were labeled: 1: 1100 to 1500 h, 2: 1500 to 1900 h, 3: 1900 to 2300 h, 4: 2300 to 0300 h, 5: 0300 to 0700 h, 6: 0700 to 1100 h.

Sleep Spindle Data

There was no significant group by period interactions on either training day 1 or 2 (data not shown), however, learning rats did have elevated spindle density over training day 1 (F(2,17) = 6.15, p = 0.011) and a change in spindle density across recording periods on training day 1 (F(7,105) = 3.00, p = 0.007). Increased spindle density persisted into training day 2 (F(2,17) = 6.07, p = 0.011), however there was no significant effect of period.

Supplemental References
