The Effects of Simple Procedural, Cognitive Procedural and Declarative Learning on Sleep

by

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Abstract

Sleep spindles have been found to increase following an intense period of learning on a combination of motor tasks. It is not clear whether these changes are task specific, or a result of learning in general. The current study investigated changes in sleep spindles and spectral power following learning on cognitive procedural (C-PM), simple procedural (S-PM) or declarative (DM) learning tasks. It was hypothesized that S-PM learning would result in increases in Sigma power during Non-REM sleep, whereas C-PM and DM learning would not affect Sigma power. It was also hypothesized that DM learning would increase Theta power during REM sleep, whereas S-PM and C-PM learning would not affect Theta power.

Thirty-six participants spent three consecutive nights in the sleep laboratory. Baseline polysomnographic recordings were collected on night 2. Participants were randomly assigned to one of four conditions: C-PM, S-PM, DM or control (C). Memory task training occurred on night 3 followed by polysomnographic recording. Re-testing on respective memory tasks occurred one-week following training. EEG was sampled at 256Hz from 16 sites during sleep. Artifact-free EEG from each sleep stage was submitted to power spectral analysis.

The C-PM group made significantly fewer errors, the DM group recalled more, and the S-PM improved on performance from test to re-test. There was a significant night by group interaction for the duration of Stage 2 sleep. Independent t-tests revealed that the S-PM group had significantly more Stage 2 sleep on the test night than the C group. The C-PM and the DM group did not differ from controls in the duration of Stage 2 sleep

on test night. There was no significant change in the duration of slow wave sleep (SWS) or REM sleep.

Sleep spindle density (spindles/minute) increased significantly from baseline to test night following S-PM learning, but not for C-PM, DM or C groups. This is the first study to have shown that the same pattern of results was found for spindles in SWS. Low Sigma power (12-14Hz) increased significantly during SWS following S-PM learning but not for C-PM, DM or C groups. This effect was maximal at Cz, and the largest increase in Sigma power was at Oz. It was also found that Theta power increased significantly during REM sleep following DM learning, but not for S-PM, C-PM or C groups. This effect was maximal at Cz and the largest change in Theta power was observed at Cz.

These findings are consistent with the previous research that simple procedural learning is consolidated during Stage 2 sleep, and provide additional data to suggest that sleep spindles across all non-REM stages and not just Stage 2 sleep may be a mechanism for brain plasticity. This study also provides the first evidence to suggest that Theta activity during REM sleep is involved in memory consolidation.

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The Effects of Simple Procedural, Cognitive Procedural and Declarative Learning on Sleep

Although the precise functions of sleep remain a topic of debate, many recent studies have focused on the role of sleep in memory and learning. This thesis will report from an experiment designed to investigate the effects of simple procedural, cognitive procedural and declarative learning on sleep. Specifically, Stage 2 sleep and sleep spindles are hypothesized to play a role in sleep-dependent learning. To provide the rationale for the study design and hypotheses, the introduction of the thesis will begin with a general overview of sleep, sleep architecture, and theories of the functions of sleep, followed by a detailed review of factors known to be related to sleep spindles. A general overview of the structures and consolidation mechanisms implicated in the various memory systems will be followed by a focused account of the relevant literature on the role of sleep in memory.

Sleep

We spend almost 1/3 of our lives asleep, yet it is not well understood *why* we sleep. One of the most longstanding perspectives is that sleep is involved in reprocessing previous waking experiences. The interpretation of dreams by artists, poets, and philosophers is likely the origin of this idea, and continues to be a source of scientific hypotheses (Stickgold, Hobson, Fosse & Fosse, 2001). The practice of "dream incubation" by the ancient Mesopotamians, Egyptians and Chinese (for review see: Van De Castle, 1994) is one of the earliest examples of the involvement of dreams in the processing of material from previous waking experiences. The ancient Roman poet Lucretius (Lucretius, trans. 1947) proposed that we continue to think about waking

experiences during sleep:

> *"Generally, ... on whatever subject we have much previously dwelt, the mind having been put to more than usual strain in it, during sleep we are engaged in the same."*

Freud, (trans, 1950) also considered dreams to be a representation of previous waking experience in what he called "day residue".

Sleep Architecture

Over the course of a night, sleep varies in terms of brain activity and behaviour in a regular rhythmic pattern (Carskadon & Dement, 2000). In young adults, the first half of the night is composed of primarily non-REM sleep (NREM) but little REM sleep. The last half of the night is composed almost entirely of Stage 2 and REM sleep whereby the periods of REM sleep lengthen as the night progresses (Figure 1). Over the course of a given night, there are four to five NREM - REM cycles. About 50 to 60% of a night's sleep is made up of Stage 2 sleep, and only about 20 to 25% is REM sleep (Hobson, 1998).

Tonic and phasic EEG and overt behaviour systematically vary over the course of the night (Rechtschaffen and Kales, 1968). Each stage of sleep is characterized by a particular EEG frequency that may predominate, however, all stages have mixed frequencies present. Alert waking EEG is characterized by low amplitude, mixed frequency EEG including alpha (8 to 12 Hz), beta (16 to 35 Hz), and gamma (35 to 70 Hz). Fast, high amplitude eye movements (EOG), high muscle activity (EMG) are also markers of wakefulness. While resting with eyes closed but still awake, the EEG becomes more rhythmic and slower. The EEG is characterized by alpha frequency activity (8 to 12 Hz). Sleep onset is characterized by changes in a number of physiological and

behavioural indices (for review see Ogilvie, Simons, Kuderian, et al., 1991; Ogilvie, 2001). In general, the transition from wakefulness to Stage 1 sleep is characterized by systematic changes in EEG and overt behaviour. For example, decreases in alpha, increases in theta activity, vertex sharp waves, slow rolling eye movements, and reduced allocation of attentional resources accompany reduced responsiveness to external stimuli (Cote, De Lugt, & Campbell, 2002).

Stage 2 sleep is characterized by the occurrence of sleep spindles or sigma activity (12 to 16 Hz), and k-complexes (Rechtschaffen and Kales, 1968). Sleep spindles are groups of distinct waxing and waning high frequency (12 to 16 Hz), relatively high amplitude (to background EEG) phasic activity that occur approximately every 4 to 10 seconds primarily throughout all NREM and especially in Stage 2 sleep. K-complexes have a well-defined negative sharp wave followed by a high amplitude positive component. The total duration of the k-complex exceeds 0.5 sec (Rechtschaffen & Kales, 1968). K-complexes occur spontaneously, but can also be evoked with auditory stimuli (Davis, Davis & Loomis, 1939; Roth, Shaw & Green, 1956). Bastien, Crowley and Colrain (2002) argue that the best method to define the evoked k-complex is by averaging to extract the waveform from background EEG. When the k-complex is averaged, its morphology consists of a biphasic negative wave (peaking at 350 and 550 ms following the stimulus, thus labeled N350 and N550), followed by a positive wave around 900 to 1200 ms (the "P900"). Bastien et al., suggest that the k-complex is indicative of lowered thalamocortical activity while others argue it is an arousal (for review see: Campbell, Bell & Bastien, 1992). Eye movements contribute little to the identification of Stage 2 sleep, and muscle activity may become lower.

Stage 3 sleep is defined by 20 to 50% delta (0.5 to 4 Hz) in a 30-second EEG epoch. Autonomic activity such as heart rate, and breathing begins to slow and becomes more regular. Stage 4 sleep has more than 50% delta activity and EOG becomes slower. Stage 3 and 4 taken together are referred to as slow wave sleep (SWS). Sleep spindles continue to occur during SWS, however, dominant delta frequencies usually masked their appearance. SWS is the deepest stage of sleep where arousal thresholds are higher than Stage 1 and 2 (but not REM) (Rechtschaffen, Hauri, & Zeitlin, 1966), and the brain's response to external stimuli is reduced relative to Stage 2 and REM sleep (Campbell, Bell, & Bastien, 1992; Tyson, Ogilvie, & Hunt, 1984).

After about 60 to 90 minutes following sleep onset, the characteristics of sleep change markedly. Tonic EEG appears to look much more desynchronized, like waking EEG, with the exception that there is usually little to no alpha activity (Rechtschaffen and Kales, 1968). Sawtooth waves mark the tracing. The most marked feature of this change in sleep is the appearance of phasic events termed rapid eye movements (REMs). During rapid eye movement (REM) sleep, the brainstem suppresses muscle movements in the major muscle groups with only a few exceptions. The muscle groups surrounding the eyes (saccadic muscle) are impervious to paralysis and thus REMs can appear. Much of the more unusual and vivid dream content is associated with REM sleep (Hobson, 1998), although dreaming occurs in all stages of sleep.

Functions of Sleep

One of the most fundamental unanswered questions about sleep is: "What are the functions of sleep?" There are multiple theories of the functions of sleep including restorative theories (Adam & Oswald, 1977), thermoregulatory (McGuinty & Szymusiak,

1990), energy conservation (Berger & Phillips, 1995), developmental theories (Roffwarg, Musio & Dement, 1966), and learning theories (See: Maquet, Smith & Stickgold, 2003 for review). Part of the difficulty in answering such a question, is that these theories are not necessarily mutually exclusive. Despite the complexity of so many theories, brain structures and mechanisms specific to sleep-related memory functions can nevertheless be isolated.

The thermogenic hypothesis states that sleep serves a number of homeostatic functions. During sleep, both brain and body temperature drop, which McGuinty and Szymusiak (1990) suggest, may serve to conserve energy, protect the brain from prolonged high temperatures during wakefulness, and facilitate immune functions. At sleep onset, body temperature drops and remains low during non-REM sleep. During REM sleep, body temperature is further inhibited (Gloztbach & Heller, 2000). One of the major criticisms of this hypothesis is that body temperature fluctuates with circadian rhythms across states of wakefulness (Czeisler & Khalsa, 2000), and like many circadian processes, sleep serves to align these rhythms. The more marked thermoregulation during sleep may simply be a part of the larger circadian factor rather than to sleep *per se*.

An obvious consequence of being less physically active during sleep is that metabolic rate is lower. The Energy conservation (Berger & Phillips, 1995) and restoration (Adam & Oswald, 1977) hypotheses are complimentary in this respect. In both cases, by reducing metabolic requirements, sleep may function to conserve energy and to allow for tissue restoration. This could be accomplished simply by rest (Berger & Phillips, 1995) or by limiting metabolism (Zepelin, 2000). Evidence that does not support these hypotheses comes from studies that have shown that sleep only reduces energy

consumption by 10 to 15 % over and above quiet wakefulness (Rechschaffen, 1998).

Because sleep varies considerably over the lifespan, from almost 50% REM sleep in infancy throughout the 16 to 18 hours spent asleep (Anders Sadeh, & Appareddy, 1995), to increasing fragmentation and reduced sleep duration throughout the lifespan, sleep may function to promote brain maturation (Roffwarg et al., 1966). The decline in REM sleep parallels the rate of brain maturation. This hypothesis is most complimentary with the sleep and learning hypothesis. The developmental hypothesis suggests that the function of sleep is not simply the consolidation of memory, but brain plasticity in general.

In this study, the findings will be interpreted within the context of the hypothesis that one of the functions of sleep is the consolidation of memory. In their book, Maquet, Smith and Stickgold (2003) provide an overview of the recent milestone experimental findings from a variety of scientific disciplines including human and animal behaviour, developmental, neural systems, cellular and molecular levels of investigation. While this collection of scientific work provides strong evidence to support the role of sleep in the consolidation of memory, the hypothesis remains open for debate and requires additional experimental testing. There are still many unanswered questions about the specific types of learning that are sleep dependent, the types of sleep that are important for each type of memory, the specific neural mechanisms involved in the consolidation of these types of memory, and their action during sleep. This study was designed to investigate these issues.

One of the first researchers to observe a relationship between learning and sleep was Ebbinghaus (See: Maquet et al., 2003, p. 2). Ebbinghaus is famous for his detailed

study of forgetting rates, and in his observations he noticed that the rate of forgetting of nonsense syllables was less when followed by an 8 hour period of sleep compared to an 8 hour period of wakefulness. This early finding is confounded by circadian effects due to the different times that training occurred across experimental conditions. Nonetheless, this was one of the first observations which suggested that a function of sleep might be the consolidation of new learning.

Since the discovery of REM sleep (Aserinski & Kleitman, 1953) in the 1950s, a number of studies used problematic experimental designs that confounded species, time of testing, sleep deprivation techniques, and problematic theoretical perspectives. A review by Crick and Mitchison (1983) is an example of research from this period where they outlined a theory of sleep and learning. They proposed that the purpose of REM sleep was to remove unwanted or unnecessary memories that would otherwise interfere with more useful memories. Their theory proved to have many flaws. They postulated that a reverse learning mechanism was responsible for determining which memories are maladaptive, or 'parasitic'. In particular, the "theory of forgetting" could not address how the brain identifies what to forget and what not to forget, and was not verified experimentally. Despite its drawbacks, many of the ideas that Crick and Mitchison suggested have been influential in formulating current theories about sleep and memory. Most importantly, they suggested that sleep is an opportune time for memories to be strengthened since both input and output from the environment is greatly reduced (Krueger & Obal, 1993). Secondly, brain activity during sleep is highly synchronized between cortical and subcortical structures (Destexhe & Sejnowski, 2001). This type of activity is consistent with what we know about how neuronal networks are

reorganized/specified to consolidate memories (McGaugh, 2000).

More recently, theories of sleep and memory have been reformulated due to a more sophisticated understanding of the cognitive and neural mechanisms of memory consolidation. Researchers now focus on the types of sleep that are necessary for memory consolidation, and the types of phasic and tonic brain activity associated with new learning. Sleep and memory theories can now be interpreted within a much richer context of consolidation theory, how neural networks function, and plasticity in the brain occurs.

Factors Related to Sleep Spindles

In order to study changes in sleep spindles it is important to consider factors that are related to the temporal and spatial variability in spindles. A number of such factors have been identified including scalp location (Jobert, Poiseau, Jahnig, Schultz, & Kubicki, 1992; Werth, Achermann, Dijk & Borbely, 1997; Zeitlhofer, Gruber, Anderer, et al. 1997), generators (Anderer, Klosch, Trenker, et al., 2001; Merica, 2000), menstrual cycle (Driver, 1996; Ishizuka, Pollack, Shirakawa, et al., 1994), age (Landolt & Borbely, 2001; Landolt, Dijk, Akermann, & Borbely, 1996; Nicolas, Petit, Rompre, Montplaisir, 2001), sleep cycle (De Gennaro, Ferrara, & Bertini, 2000; Himanen, Virkkala, Huhtala, & Hasan, 2002), and intelligence (Fogel & Smith, 2003; Nader & Smith, 2003). A more detailed discussion of these findings follows.

In a comprehensive study of sleep spindles, De Gennaro, Ferrara & Bertini (2000) investigated the topographical distribution of spindles in either SWS or Stage 2 sleep. They found that sleep spindles were maximal over centro-parietal midline derivations (Cz and Pz) compared to other midline derivations in both Stage 2 sleep and SWS. In addition, they investigated intra-cycle variations in spindles during Stage 2 sleep and

changes in spindles across sleep cycles. They found that spindle density increased over the course of the night, and that this was primarily due to variations in Stage 2 sleep spindles at centro-parietal midline derivations.

It has also been shown that slow spindles (11.5 - 14 Hz) have an anterior distribution while fast spindles (14 - 16 Hz) have a posterior distribution (Jobert et al., 1992; Werth et al., 1997; Zeitlhofer, et al., 1997). In a recent study, this phenomenon has been investigated in greater depth using more qualitative methods (Doran, 2003). The topography of slow (11 - 13Hz) and fast (13 - 15Hz) sleep spindles were compared on their propagation over time. Doran found that slow spindles were distributed over areas that are more widespread and frontal than fast spindles, whereas fast spindles were distributed centro-parietally and more localized. These results support the proposition that slow and fast spindles originate from different generators (Merica, 2000) and that they may be functionally dissociable. Alternatively, the low spindle band may contain overlapping frequencies with alpha activity.

Spindles have also been found to vary across the menstrual cycle (Driver, 1996; Ishizuka, et al., 1994). In a very comprehensive study by Driver (1996) on the power frequency variations of sleep within subjects across the entire menstrual cycle, she found that the power in the 14.25 to 15.0 Hz band peaked at the mid luteal phase. However, it was also found that the variation in sigma power covaried with body temperature. The increase in sigma power could have been due to the fluctuations in body temperature, and not hormone changes with the menstrual cycle. In addition, this finding could be spurious considering it was the only major variation in the entire power spectra band over the course of the menstrual cycle and was found only in a less than 1 Hz frequency bin. This

relationship would not be expected to account for much inter-individual differences in spindles or be expected to interact with changes in spindles related to their function.

While it is known that sleep quality and quantity change over the lifespan, of interest to this study are the age-related changes associated with Stage 2 sleep and sleep spindles. Landolt and Borbely (2001) compared power spectral differences in young vs. middle-aged men in a cross-sectional study. They found that overall, sleep efficiency and total sleep time decline with age. In addition, it was found that the duration of Stage 2 sleep and power in the lower-sigma band (12 Hz) was lower in middle-aged men (Landolt & Borbely) in groups with equivalent amounts of Stage 2 sleep (Landolt et al., 1996). Age-related decreases in sigma power were associated with a shift in peak power from frontal to central sites. These results suggest that with age, slow sleep spindles decrease in either number, duration, or amplitude and are distributed less frontally compared to younger adults.

In a more detailed investigation of the age-related decrease in sleep spindles, Nicolas, Petit, Rompre and Montplaisir (2001) divided participants into 6 age groups spanning 10 years from age 10 to 69. They found that the amount of Stage 2 sleep, the number, density and duration of sleep spindles decreased gradually with age. Conversely, sleep spindle frequency and inter-spindle interval was found to increase with age. The results support Landolt and Borbely's (2001) findings and in addition, provide a more continuous examination of the decrease in sleep spindles over the lifespan. Furthermore, they found that the higher spindle frequencies increase with age. These results taken together with Landolt and Borbely's findings suggest that slower spindle frequencies decline, yet higher spindle frequencies increase with age. These findings could have

important implications for the interpretation of the changes in spindles as a result of learning (age and learning-dependent changes in sleep spindles could interact). For this reason, all participants in the current investigation were young adults.

Silverstein and Levy (1975) conducted one of the first studies to investigate intra-individual variations of sleep spindles. The number of Stage 2 sleep spindles was found to have a curvilinear distribution across the night: starting low at the beginning of the night, peaking in the middle of the night, and dropping off at the end of the night. De Gennaro, Ferrara and Bertini (2000) found that spindle density (number of spindles/time in Stage 2 sleep) during NREM sleep increased over the course of the night and had a centro-parietal distribution. In addition, the frequency of sleep spindles has been found to vary across sleep cycles (Himanen, et al., 2002). Himanen et al. found that within each of the first four sleep cycles spindle frequency followed a curvilinear u-shaped pattern. The frequency of sleep spindles started off around 13 to 14 Hz at the beginning of each cycle and by the middle of the cycle spindle frequency reached a minimum of 12 to 13 Hz. By the end of the cycle, spindle frequency returned to 13 to 14 Hz. Therefore, higher frequency spindles were associated with stage transition. In the fifth sleep cycle, spindle frequencies were more stable across the cycle that ranged on average within 13 to 14 Hz. This may have been related to reduced sleep pressure at the end of the night, or greater homeostatic pressure for REM sleep at that time of night.

Sleep spindles vary across the sleep cycle. One of the reasons for this variation is the inverse relationship between sleep spindles and delta activity (Aeschbach & Borbely, 1993; Dijk, Hayes & Czeisler, 1993; Uchida, Maloney, March, et al., 1991). Slow wave activity is highest in early sleep and decreases as sleep progresses (Aeschbach, Dijk &

Borbely, 1997). On the other hand, spindle activity increases with sleep (Aeschbach & Borbely, 1993). Functionally, slow wave activity is considered a marker of NREM sleep intensity, and serves to maintain sleep homeostasis (Borbely, 1982). It has been suggested that slow wave activity and sleep spindles are generated by a common thalamocortical system (Steriade and Amzica, 1998; Steriade, McCormick & Sejnowski, 1993). Slow wave activity and sleep spindle oscillations may be incompatible with one another. Thus, an inverse relationship between slow wave activity and sleep spindles can be observed across NREM sleep, especially when slow wave activity is very high, for example in early NREM sleep episodes (Aeschbach & Borbely, 1993).

While sleep spindles in NREM appear to vary over the course of the night, there are also great inter-individual differences in sleep spindles (Roth, Shaw & Green, 1956; Werth, et al., 1997). Until recently there has been little explanation for the individual differences in the number of sleep spindles. Gibbs and Gibbs (1962) observed that individuals with mental retardation have unusual spindles that they termed "extreme spindles". These spindles may reflect dysfunction in the gating mechanisms in thalamocortical loops producing these extreme spindles. They reported that 70 to 80 percent of mentally retarded children (well below average intelligence) observed exhibited this pattern of abnormal brain activity. Recent findings have suggested that spindle activity during sleep may be related to intelligence (Fogel & Smith, 2003; Nader & Smith, 2003). Specifically, Nader and Smith (2003) found that the number, density and power spectra of Stage 2 sleep spindles was related to Performance and Full scale IQ but not Verbal IQ. Fogel & Smith (2003) found that IQ accounted for about 75% of the inter-individual variability in sleep spindles. These findings suggest that spindles may be a

biological marker for efficiency of information processing in thalamocortical networks, and that they account for much of the inter-individual differences in spindles. Alternatively, if spindles are related to processing of new learning, it may be that a greater amount of learning (or perhaps more efficient learning) takes place in a higher IQ group.

Memory Systems

An important development in sleep and memory research has been largely due to new ways to operationalize memory. In the past, studies of sleep and memory have had mixed results because of the choice of memory tasks. Now, using finer distinctions for defining the type of memory and the nature of the tasks, researchers can study the relationship between sleep and memory with greater precision. The brain structures and consolidation mechanisms for each type of memory will be discussed. This review of the developments in memory research is intended to provide an appropriate background for understanding the relationship between sleep and memory.

While the taxonomy of memory has been extensively researched in both cognitive and behavioural neuroscience, and many of the associated brain structures have been localized due to the advent of imaging and source localization techniques, it is not entirely clear what physiological mechanisms are involved in memory consolidation. Memory consolidation is the process by which memories in the neocortex initially formed by short-term memory are strengthened into long-term storage. This process is thought to primarily involve brain structures such as the hippocampus and the thalamus. Over the course of consolidation, the influence of the hippocampus or the thalamus diminishes until the memory is permanently stored in the neocortex (Ivanco & Racine, 2000;

Winocur, Moscovitch, Fogel, Rosenbaum & Sekeres, submitted). Amnesic patients have been studied extensively to better understand the systems that make up human memory (see Nadel & Moscovitch, 1997 for review). Animal models have been used to manipulate the factors that contribute to deficits observed in amnesic patients, to pinpoint the brain structures involved, and to better operationalize associated memory deficits (see Rosenbaum, Winocur, & Moscovitch, 2001 for review). As a result of this work, many of the brain structures and processes involved in the long-term storage of declarative memory have been identified including the perirhinal cortex, the parahippocampal cortex, the entorhinal cortex, the dentate gyrus, the hippocampus, and the subiculum (Squire & Zola-Morgan, 1996). Declarative memory is often thought of as "knowing what", that is defined as knowledge for facts that require conscious recollection. Episodic memory is a type of declarative memory for events or occurrences that are usually dependent on the context in which the memories are formed. On the other hand, procedural memory is thought of as "knowing how", that is defined as memory for procedures that must be performed that are independent of conscious recollection and are learned implicitly (Squire, 1998; Zola-Morgan & Squire, 1993).

Interestingly, procedural memory is spared in amnesia resulting from damage to the medial temporal lobe. Thus, it is thought that brain structures outside the medial temporal lobe are involved in the consolidation of procedural memory. Procedural memory is dependent on a variety of structures of that are independent of the hippocampus. The compartmentalization of the types of procedural memory to different brain structures suggests that there may be separate mechanisms for consolidating each subtype of memory. Sleep plays an important role in the consolidation of memory

(Maquet, Smith and Stickgold, 2003), and the study of sleep-related EEG is a useful way to study the brain mechanisms involved in memory consolidation.

Declarative Memory

Declarative memory involves conscious recollection of memories for facts and events (Squire & Knowlton, 2000). There is an underlying consensus on the major types of memory (Squire & Knowlton, 2000; Schacter & Tulving; 1994) despite the varying terminology used in the literature. A major focus of current memory research is to identify the subtypes of memory and the associated brain structures and the mechanisms involved in the consolidation of memories into long-term storage.

Brain structures. The best evidence indicating that memory is consolidated into long-term storage over long periods of time comes from animal models of amnesia (since pre-injury memory performance in human models is usually retrospective in nature). Retrograde amnesia is temporally graded; memories closer to the time of injury are more impaired than distant memories (Kim, Clarke, & Thompson, 1995; Kim & Fanselow, 1992; Winocur, 1990; Zola-Morgan & Squire, 1990). Evidence to support the idea that recent memories are consolidated over long periods of time comes from the finding that remote memories are less susceptible to impairment than recent memory. Complete bilateral lesions to the hippocampus administered at various delays following equivalent amounts of training on a context-dependent memory task impair subsequent memory performance (Winocur, 1990). Animals with brain lesions administered closer to initial learning have more severe memory impairment than animals with longer intervals between initial learning and brain lesion. Similar results have been observed when lesions are localized to the thalamus (Winocur, 1990), the entorhinal cortex (Cho, Beracochea &

Jaffard, 1993; Cho & Kesner, 1996), and the fornix (Wiig, Cooper & Bear, 1996). These findings suggest that many of the structures in the medial temporal lobe are involved in memory consolidation over long periods of time.

With the advent of imaging techniques such as Computerized Axial Tomography (CAT), Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI) structures involved in memory consolidation, storage, and retrieval can be identified with high spatial resolution using noninvasive techniques. During a widely used task to assess declarative memory such as the paired-associates task, Mottaghy, Shah, Krause, et al. (1999) found that prefrontal brain activation was asymmetrical during encoding and retrieval of paired-associates learning. These findings were supportive of Tulving et al, (1994) meta-analysis of 14 PET studies on encoding and retrieval of episodic memory. In addition, they found that the hippocampus, cerebellum, and thalamus were also involved in memory retrieval. These findings suggest that the cerebellum, thalamus, hippocampus and the prefrontal cortex are important for declarative memory functions.

Consolidation mechanisms. The medial temporal lobe has been thought of as a temporary location for memories to be moved into long-term storage in the neocortex. The medial temporal lobe is thought to be less involved in memory consolidation over time, as memory traces in the neocortex become stronger (Nadel & Moscovitch, 1997; Rosenbaum, Winocur, & Moscovitch, 2001; Squire & Knowlton, 2000). Memories are thought to become permanently stored in the neocortex, eventually becoming independent of the medial temporal lobe (Winocur, et al., submitted). Traditionally, the mechanisms for this consolidation are thought to involve Hebbian-type principles (Hebb,

1966) over networks of neurons in the neocortex (McClelland, Rumelhart & the PDP Research Group, 1988). Long-term potentiation (LTP) is thought of as one of the mechanisms involved in the consolidation of memory (Chapman, Trepel, Ivanco, et al, 1998; Ivanco & Racine, 2000; Trepel & Racine, 1998). LTP of synaptic pathways occurs to networks of neurons both pre- and post-synaptically. It is the process by which dendritic growth increases, synaptic distance decreases, neurotransmitter reuptake increases, and axonal conduction becomes more efficient by increased mylenation (Gustafson & Wigstrom, 1988).

Non-declarative Memory

Non-declarative memory involves performance on procedures, habits, conditioning and priming that is usually independent of conscious retrieval. It is often considered knowing or learning "how" as opposed to knowing or learning "what" that is usually associated with declarative memory. Non-declarative memory is usually further subdivided into motor (or simple) procedural memory, and cognitive (or complex) procedural memory. For example, the pursuit rotor has been classified as a simple motor procedural memory task, whereas the mirror tracing task has been classified has been classified as a cognitive procedural memory task (Smith, 1995; 2001).

Brain structures. Memory for skills and habits has been found to be associated with the striatum; priming and perceptual learning is dependent on the neocortex; simple classical conditioning involves the cerebellum; and nonassociative learning is dependent on reflex pathways (Squire, 1998). The compartmentalization of memory is a useful theoretical heuristic. However, theories using this research paradigm are simplistic in light of the apparent sophistication and integrative nature of neuronal networks. Despite

this, some brain structures repeatedly stand out as serving a more central coordinating role in the consolidation of memory. The thalamus has been suggested as one possible structure that may be an analog to the hippocampus playing a role in earlier stages of memory consolidation (Winocur, 1985).

Using PET imaging of cerebral blood flow, Grafton, Mazziotta, Presty, et al. (1992) investigated the changes in cerebral blood flow during the acquisition of simple motor procedural learning. Grafton et al. found that the primary motor cortex, supplementary motor area, and the pulvinar of the thalamus were more active during task acquisition over the 75 minute training session relative to controls. These findings indicate that the areas involved in the execution of motor plans are also active during the initial stages of learning. In a second study with a larger sample, Grafton, Woods, and Tyszka (1994) found that the same areas of the brain were active during pursuit rotor learning. In addition to the previous findings, the cerebellum was also found to have increased blood flow. To investigate the changes in brain activity over the long-term course of task acquisition, participants returned 48 hours later and repeated the same number of trails as on day 1. They found that changes to cerebral blood flow were greatest in putamen, parietal cortex and premotor cortex. These findings suggest that at first during the early stages of procedural learning, brain activity increases in the cortical motor areas, cerebellum and thalamus. However, over longer intervals, execution of the learned motor plan no longer involves the thalamus and the cerebellum.

Recently, Balslev, Nielsen, Frutiger, et al. (2002) investigated changes in brain activity associated with more cognitively complex forms of procedural learning. They used PET imaging to identify the pattern of brain activity associated with new cognitive

procedural learning. It was found that the premotor, supplementary motor and posterior parietal cortex were more active during task acquisition. Grafton, Mazziotta, and Presty (1992) also found that increased activity resulted from mirror tracing in the middle frontal gyrus, supplementary motor area, basal ganglia and cerebellum during learning. Together, these findings indicate that areas involved in the execution of motor behaviour including the frontal cortex and basal ganglia are involved in the consolidation of more cognitively complex memory. In other words, the motor execution areas appear to be involved in simple procedural memory, whereas the frontal cortex and the basal ganglia are needed in addition to these areas for the consolidation of cognitive procedural memory.

Consolidation mechanisms. It is known that the long-term storage of procedural memory requires repeated trials or practiced learning and occurs primarily in the neocortex and is dependent on thalamo-cortical oscillations. This type of memory encoding requires spaced and repeated stimulation for LTP to occur (Chapman et al., 1998; Trepel & Racine, 1998). The thalamus has been proposed as one structure that may be of particular importance for procedural learning (Asanuma & Palvildes, 1997; Gilbert, 2001). Projections from the thalamus to the neocortex and back to the thalamus form thalamocortical loops that fire in an oscillatory pattern. The oscillatory activation of these circuits has been implicated as one of the mechanisms for LTP (Gilbert, 2001). The coalescent activity of thalamocortical loops results in sleep spindles during sleep. The thalamus receives input from the hippocampus, the cerebellum and basal ganglia. Gilbert (2001) proposes that input from the hippocampus is thought to be involved in conscious recollection via cortical layer I neurons. With reactivation, the connections between the thalamocortical loops and layer 2/3 neurons become stronger by long-term potentiation

until recall is independent of the hippocampus. Gilbert also posits that procedural memory is formed in a similar way, with the exception that the thalamus receives input from the basal ganglia and cerebellum as opposed to the hippocampus (Figure 2).

Gilbert suggests that skilled motor movements requiring conscious thought involves contextual input to thalamocortical units, whereas automatic motor movements require input from the cerebellum to thalamocortical units. Steriade (1999) has shown that the reactivation of thalamocortical circuits is particularly synchronized during sleep, especially during Stage 2 sleep in the form of sleep spindles. Sleep spindles have been shown to increase as a result of new learning (Fogel, Jacob & Smith, 2002), and are thought to be one of the mechanisms that induce long-term potentiation in the brain (Destexhe & Sejnowski, 2001).

Sleep and Memory

Behavioural and electrophysiological studies of humans and animals have been carried out to test the hypothesis that one of the functions of sleep is memory consolidation (for review see: Maquet, Smith and Stickgold, 2003). Two main paradigms have been used in humans and animals to investigate the relationship between sleep and memory. These include sleep deprivation and recording studies. The sleep deprivation paradigm has been used extensively to assess the effects of sleep loss on memory by measuring pre-post differences in memory performance with an intervening period of total or selective sleep deprivation. One of the shortcomings of this paradigm is that it is usually not known whether the deterioration in performance following sleep deprivation is limited to memory *per se*. Thus, it is important not only to test the specific types of learning and other cognitive function that are affected by sleep deprivation, but also to

investigate the learning-dependent changes in sleep following new learning. This approach provides insight into the mechanisms involved in the consolidation of memory during sleep. The recording paradigm has been used to observe changes in sleep architecture and changes in tonic and phasic EEG following learning on a memory task. The changes in these sleep parameters are often compared to baseline sleep, and sleep in non-learning controls. This method is used to assess changes in brain activity during sleep as a result of new learning. In order to establish that sleep affects learning and learning affects sleep with respect to a particular stage of sleep and type of memory, evidence from both paradigms must be obtained.

Animal Research

Much of the groundbreaking research in the area of sleep and memory was conducted on animals (See Smith, 1985; 1995 for review). The focus of much of this research has been on REM sleep. One of the major developments in this area was the description of the REM sleep window (RSW). The RSW is characterized by an increase in the duration of REM sleep following new learning or density of REMs (Smith & Lapp, 1986; Smith & Rose, 1997b). When REM sleep deprivation is applied during the critical period known as the RSW, memory performance is impaired compared to normally rested controls. If the same amount of REM sleep deprivation is applied at a time outside of the RSW, memory performance is no different from rats that have slept uninterrupted. REM sleep deprivation does not necessarily have to last for the entire duration in the RSW for memory impairment to occur. Rather, disruption of REM sleep during the expected RSW is sufficient to impair performance. The RSW varies as a function of strain of the animal (Smith, Lowe & Smith, 1977), the type of memory task (Smith &

Rose, 1997a; Smith, Young & Young, 1980), and the training schedule (Smith, Young & Young, 1980). Both the total number of trials and the number of trials per session will cause the timing of the RSW to vary. Three studies examined the effect of varying the training schedule of the two-way shuttle avoidance task on the timing of the RSW. In the first study, 100 trials were administered in one single training session (Smith et al., 1980). In the second, 50 trials per session were administered over 2 sessions (Smith & Lapp, 1986), and the third, 20 trials per session were administered over 5 sessions (Smith et al, 1980). Interestingly, for the distributed trials, the largest increase in REM sleep was observed in the group that was administered all the trials in one training session. This suggests that an intense period of learning in a single session necessitates the largest changes in sleep. For this reason, in this study learning training on the memory tasks was conducted in a single testing session. Interestingly, the largest increase in REM sleep was observed in the 24-hour period prior to their maximum increase in correct performance. In addition, rats that did not learn had comparable amounts of REM sleep over baseline as non-learning controls. In order to determine the exact timing of the RSW, short periods of REM sleep deprivation (4 hours) were administered throughout the period following the last training trial. When rats were trained with 100 trials per session, a RSW was found at 1 to 4 hours port training. When training was distributed over two sessions, two RSWs were found at 9 to 12 hours after training, and 53 to 56 hours after training. When training was more distributed into 5 sessions of 20 trials, the same 9 to 12 hour RSW was found, and the second at 17 to 20 hours post-training. RSWs have also been found using a number of other memory tasks including the Morris water maze (Smith & Rose, 1997a; 1997b), the radial arm maze (Smith, Conway & Rose, 1998), and the conditioned cue

preference task (Vallance, McDonald, & Smith, 1999).

More recently, there has been an effort to better define the RSW in animal studies. The neurotransmitter acetylcholine is known to be important for both REM sleep (Smith, 2003) and memory (Hasselmo, 1999). In one study, scopolamine (an acetylcholine agonist) was infused into the lateral amygdala to suppress cholinergic activity at 6, 9 or 12 hours after training to coincide with the RSWs specific to the memory task, training schedule, and species used in this experiment (Smith, 2003). Memory for the conditioned cue preference task was impaired relative to rats that received saline injections compared to scopolamine injections at 6 or 12 hours post-training. Thus, when scopolamine was infused into the amygdala (that is known to be involved in the conditioned cue preference task (McDonald & White, 1993) during a known RSW, memory performance was impaired. This research indicates that cholinergic activity in the lateral amygdala is important for memory consolidation during REM sleep in rats.

Human Research

Both REM sleep and Stage 2 sleep have been found to be involved in human memory performance. REM sleep is involved in the consolidation of cognitive procedural memory, and Stage 2 sleep is involved in the consolidation of simple procedural memory (Tweed et al., 1999). Only a limited number of studies report that either REM or Stage 2 sleep are involved in declarative memory consolidation (Barrett & Ekstrand, 1972; Gais, Molle, Helms & Born, 2002). The following sections will discuss these findings in greater detail.

REM sleep and cognitive procedural memory. There is a great deal of evidence to suggest that REM sleep is involved in procedural learning, and only limited evidence

to suggest that REM sleep is involved in declarative memory or other types of memory (Smith, 2001). 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 trampolining training program as the novel motor skills learning task. This task requires an individual to make translation and rotations of the whole body in three-dimensional space. After eight separate training sessions, polysomnographic recordings of the participant's sleep were recorded. Their sleep parameters were compared to their own baseline values taken seven days before training. It was found that there was an increase in the minutes of REM sleep, and changes in the NREM/REM sleep cycle after learning. These findings suggest that new cognitive procedural learning is dependent on REM sleep. However, it was not clear whether the increase in REM sleep was due the need for physical restoration after physical activity.

A second study was conducted to determine if the changes in REM sleep were a result of new learning or simply physical activity (Buchegger, Fritsch, Meier-Koll, Riehle, 1991). Again, the trampolining training program was used as the learning condition while controls either danced or played soccer. It was found that the amount of REM sleep increased following trampolining compared to baseline and physical activity controls. These findings supported the original hypothesis that REM sleep is important for cognitive procedural memory, and suggest that REM sleep did not fluctuate simply as a result of physical activity alone.

In one of the few studies of its kind, Smith and Weeden (1990) found that presentation of a clicking noise coincident with rapid eye movements in the REM sleep state, also present during initial training improved subsequent task performance. The

logic task used in this experiment was previously found to be impaired due to REM sleep deprivation (Lapp & Smith, 1986; Smith, 1993) which suggests that REM sleep and rapid eye movements during REM sleep may be involved in the consolidation of cognitive procedural memory. However, this hypothesis remained to be tested experimentally. The participants were asked to work on the logic task for 45 minutes during which an auditory clicking noise was present in the background. This noise was not explicitly pointed out to any of the participants. On the night following the training session, a similar clicking noise was administered coincident with rapid eye movements. It was found that when the clicking noise was administered coincidentally with rapid eye movements, task performance was improved upon re-test relative to the group without the clicking noise present. These results indicated that REM sleep is involved in procedural memory that requires complex logic skills, and that rapid eye movements themselves are involved in the consolidation of cognitive procedural memory. These findings suggest that rapid eye movements may increase following new cognitive procedural learning.

Most recently, Smith, Nixon and Nader (manuscript submitted for publication) conducted an experiment to determine if this hypothesis was tenable. They used well-established cognitive procedural tasks, the mirror tracing task and the Tower of Hanoi to determine whether rapid eye movements increase with new learning. A covariate that has attracted some attention in the sleep and learning literature was also investigated in this study. Learning potential, as measured by IQ was used to determine if the effect of learning on REM sleep differed based on IQ. Participants in the learning condition were all trained on both tasks, and subsequently split into low, medium and high IQ groups. The control group was not trained on a learning task, and was composed of individuals in

the low, medium and high IQ range. Participants were retested one week later on the Mirror Trace and Tower of Hanoi. It was found that all participants improved from training to retest on time to complete the tasks and number of errors. Furthermore, participants in the low, medium and high IQ groups differed on task performance during training. For the Mirror Tracing task, the low IQ group on average took longer to perform each trial, and made more errors than the medium and high IQ groups. The medium and high IQ groups performed at equivalent levels. For the Tower of Hanoi, the low IQ group took longer to perform the task than the medium IQ group, and the medium IQ group took longer than the high IQ group. In addition, the low IQ group required more moves to complete the task than the high IQ group, and the medium IQ required more moves than the high IQ group. Most importantly, the number of rapid eye movements and the density of rapid eye movements were higher in the participants who were trained on the cognitive procedural tasks compared to controls on the night following training. In addition, each IQ group was higher than controls following learning, and the high IQ group had an increase in rapid eye movements over their own baseline number of rapid eye movements before training. A similar pattern of results was found for rapid eye movement density with the exception that the low IQ group did not have higher REM density following learning than controls. This study has demonstrated that REM sleep is involved in the consolidation of complex procedural learning. More specifically, rapid eye movements may be a mechanism for, or somehow reflect sleep-dependent learning.

Yaroush, Sullivan and Eksrtand (1971) investigated the effect of a post-training retention interval filled with either early or late sleep on memory performance. This paradigm takes advantage of the fact that early sleep has higher levels of SWS and lower

levels of REM sleep, whereas late sleep is composed mostly of REM sleep and has little SWS. The proportion of Stage 2 sleep remains relatively constant throughout the night. In this testing paradigm, at least two experimental groups are used. One group is trained before sleeping for the first half of the night, and is retested during the second half of the night. The other group is trained during the first half of the night, sleeps for the second half of the night, and is re-tested upon awakening. Plihal & Born, (1997) found that a retention interval with a high proportion of SWS early in the night was associated with improved declarative memory performance on a paired-associates task. In addition, a retention interval filled with a high proportion of REM sleep late in the night was associated with improved cognitive procedural memory performance on the mirror tracing task. Waking control groups were used that remained awake during the retention interval to control for circadian effects *between*-groups. Nonetheless, the most interesting comparison between early and late sleep retention intervals *within*-groups is still confounded by circadian effects, and sleep inertia effects. In addition, it is not clear that time-of-night differences in Stage 2 sleep are controlled for using this design. As described above, sleep spindles change across the NREM sleep and across the night. In addition, arousal thresholds, homeostatic pressure and circadian phase all change over the course of the night. Thus, effects attributed to early or late sleep cannot be attributed solely to either SWS or REM sleep respectively. One study has addressed this issue, by either selectively depriving individuals of either REM or by disrupting Stage 2 sleep (Tweed, et al., 1999). It was found that REM deprivation impaired performance on the mirror-tracing task, but not on the simple-tracing task relative to those that were exposed to Stage 2 sleep disruption or normally rested controls. On the other hand, Stage 2 sleep

disruption impaired performance on the simple-tracing task but not the mirror-tracing task relative to REM deprivation or normal sleep. These results indicate that REM sleep is involved in the consolidation of procedural memory skills that require cognitive translations, while Stage 2 sleep is involved in the consolidation of procedural skills that have very simple cognitive attributes. As noted earlier, the use of sleep deprivation paradigms are useful for establishing a relationship between the type of memory and type of sleep related to changes in performance, however, further investigation is required to determine what changes occur during sleep as a result of learning to firmly establish a connection between sleep and memory consolidation.

Recently, Peigneux, Laureys, Fuchs, et al., (2003) conducted a study to demonstrate changes in regional cerebral activation using Positron Emission Tomography (PET) following the acquisition of a sequential artificial grammar during REM sleep. The basic premise of the use of brain imaging paradigms to test sleep-memory hypotheses is to determine if sleep plays a role in the reprocessing of information learned during wakefulness. This would be accomplished if it can be shown that the same brain structures that are activated during training are re-activated during sleep compared to a control group. The task used in this study (Cleermans & McClelland, 1991) required participants to press one of six keys as fast and as accurately as possible that corresponded to the position of one of six stimuli displayed on a computer screen. The sequence of the stimuli was determined by an artificial grammar that was not known to the participants, nor were they told that there was any pattern to the presentation of the cues. Another group of participants was tested using the same apparatus and task procedures with the exception that the sequence of the stimuli was random. 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. Four groups were used: The first group was scanned both while being trained on the serial reaction time task that used the artificial grammar and scanned during resting wakefulness. This provided a list of brain structures that were involved in task acquisition. A second group was scanned during task acquisition, and then again during wakefulness, Stage 2, SWS and REM sleep the following night. Here, the comparisons were used to determine the changes in REM sleep that resulted from training on the artificial grammar. A third group underwent the same testing and scanning protocol, with the exception that the serial reaction time task used a randomly generated sequence as opposed the artificial grammar. The comparisons made in this group would identify the brain structures more active during REM sleep than wakefulness following visuomotor learning. The fourth group was scanned under the same conditions as the second and third groups, except they were not trained on any memory task. This group provided a comparison between the post-training REM sleep and more typical REM sleep. It was found that the bilateral cuneus and adjacent striate cortex, left premotor cortex and mesencephalon were activated during both the practice session and during post-training REM sleep for the groups that performed either the sequential grammar task or the random task more than controls that did not perform the task. Moreover, it was found that the activation in the right cuneus was greater for the group that was trained on the serial reaction time task that used the artificial grammar compared to the group that was trained using the random stimulus presentation. From these findings, it was concluded that the cunues is involved in the reprocessing of the artificial grammar during REM sleep, and not simply the acquisition

of the visuomotor skills. The cuneus was previously found to be active during the acquisition of implicit sequence learning during wakefulness (Schubotz & Von Cramon, 2001). The striatum is thought to play a more critical role in the processing of artificial grammars (Grafton, Hazeltine & Ivry, 1995), and was found to be specifically related to reprocessing of artificial grammar during REM sleep in a previous study (Peigneux et al., 2000). These findings suggest that the interplay between the cuneus and striatum may implicate the basal ganglia in the consolidation of complex procedural material during REM sleep. One of the major drawbacks of this study is that participants were not randomly assigned to groups. The participants assigned to the sequential group were more rigorously screened for the ability to sleep while being scanned, whereas the random group did not meet the criteria set for sleep quality during testing. Thus, sleep quality could have confounded the reactivation observed during REM sleep.

Stage 2 sleep and simple procedural memory. The first study to investigate the relationship between Stage 2 sleep and simple procedural memory was by Smith and MacNeill (1994). They deprived four independent groups of REM sleep, non-REM sleep, total sleep deprivation or the last half of the night only. It was found that following 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 of Stage 2 sleep and REM sleep, and pursuit rotor performance was unaffected by selective REM deprivation, by deduction it was concluded that Stage 2 sleep must be important for the consolidation of simple procedural memory.

In another study investigating the relationship between the amount of Stage 2

sleep and simple procedural memory, Smith and Fazekas (1997) used a similar paradigm and deprived participants of either sleep for the entire last half of the night (regardless of stage of sleep) or just 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 and the selectively 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 from Stage 2 sleep interruption. These results indicate that even a subtle Stage 2 sleep interruption can impair simple procedural memory.

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 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. On the other hand, REM deprivation impaired performance on the mirror-tracing task compared to normally rested controls or Stage 2 sleep interruption. These findings suggested that selective Stage 2 sleep deprivation impairs simple procedural memory, but not cognitive procedural memory.

In addition to the pursuit rotor and simple tracing task, another task has been used to investigate the effects of a post-training retention interval filled with sleep. Karni, Meyer, Rey-Hipolito, et al. (1995) observed performance improvements on a finger tapping task after an intervening 24 hour period between training and retesting despite no additional practice. 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 at 10:00 AM. They performed 12 trials, 30 seconds in duration whereby they were instructed to repeat the sequence 4-1-3-2-4 as quickly and as accurately as possible by pressing keystrokes assigned to each finger. 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 daytime 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 related motor activity during this period. This group showed the same small performance improvement following the daytime 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 slow increase in performance on the finger tapping task. A large increase in performance was observed when participants were trained in the evening at 10:00 PM and re-tested at 10:00 AM. However, when re-tested again at 10:00 PM with an intervening period of wakefulness, no increase in performance was observed. This finding suggests that it is not simply the passage of time that results in performance gains on the finger tapping task, rather this 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.

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 sleep spindles. Sleep spindles have been suggested to be a likely mechanism for the consolidation of new learning (Steriade & Amzica, 1998; Steriade, 1999; Destexhe & Sejnowski, 2001). 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 CA++ ions into cortical cells. The influx of calcium ions is suggested to be a mechanism for LTP of cortical cells (Ghosh & Greenberg, 1995). This influx of calcium may serve to prime the synapses for permanent changes (Destexhe & Sejnowski, 2001). It has been proposed that this state may also be involved in the expression of genes related to synaptic plasticity (Li, Llopis, Whitney, Zlokarnik & Tsien, 1998).

While it is now clear that REM sleep deprivation impairs cognitive procedural memory, and Stage 2 sleep deprivation impairs simple procedural memory, only one study has investigated the effects of simple procedural learning on tonic and phasic Stage 2 sleep. Fogel and Smith (2002) investigated the effects of simple procedural learning on Stage 2 sleep and sleep spindles (using a variety of simple procedural learning tasks; the pursuit rotor, simple tracing task, bilboquet, and operation). It was found that the number of sleep spindles, spindle density (spindle 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 REM sleep were not affected by simple procedural learning. They concluded that Stage 2 sleep is important

for the efficient consolidation of simple procedural memory, and sleep spindle activity is one of the mechanisms for this type of memory consolidation. This was the first recording study conducted to test the hypothesis that simple procedural learning affects Stage 2 sleep. One of the major shortcomings of this study was that a combination of tasks was used which involved the refinement of both gross and fine motor control. Thus, it was not possible to determine precisely the type of learning that was related to the increase in sleep spindles. In addition, several factors were not investigated at all. The change in sleep spindles and Sigma power was not investigated in sleep stages other than Stage 2 sleep. Thus, it could not be determined whether the change in spindles was isolated to Stage 2 sleep, or continued throughout NREM sleep. In addition to the lack of data during NREM and REM sleep, frequency bands outside of the 12 to 14 Hz range were not analyzed. Thus, it was not possible to determine if the increase in Sigma power was isolated to the 12 to 14 Hz range or a global increase in power across all bands, or bands other than Sigma. Finally, fast spindles in the 14 to 16 Hz range have been suggested to originate from a separate generator than slower 12 to 14 Hz spindles. In addition, they have distinct topographical distributions (Werth et al. 1997; Zeitlhofer et al. 1997) and may thus have distinct memory related functions.

Sleep and declarative memory. One of the first well controlled studies on sleep and memory investigated the effects of memory performance after an interval filled with mostly Stage 4, REM sleep or no sleep in three independent sleep conditions (Barrett & Ekstrand, 1972). The results indicated that performance on a paired-associates task was better when the retention interval (time between pre and post-test) 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 memory. These findings suggest that sleep that consisted mostly of NREM sleep was more beneficial to declarative memory than sleep with REM sleep. Prior to this study, the group that was trained on the memory task followed by an interval of REM from the second half of the night also had an interval of early sleep immediately preceding performing the memory task. Grosvenor and Lack (1984) argued that these studies (Barrettt & Ekstrand, 1972; Ekstrand, Sullivan, Parker, & West, 1971; Fowler, Sullivan, & Ekstrand 1972; Yaroush 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 vs. 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. Before the retention interval, participants were trained on a declarative paired-associates memory task. Confirming the work done previously by Ekstrand et al, 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 vs. late sleep testing paradigm, these findings do suggest that sleep is involved in the consolidation of new memories. Declarative memory is initially hippocampal dependent (Winocur, 1990). Animal studies have shown that hippocampal dependent memory can be acquired quickly (Racine, Milgram & Hafner, 1983), and has a faster rate

of decay than in the neocortex (Trepel & Racine, 1998). However, more lasting storage of declarative memory requires slower hippocampal-cortical transfer of information (Nadel & Moscovitch, 1997; Rosenbaum, Winocur & Moscovitch, 2001; Zola-Morgan & Squire, 1990). It has been suggested (Born & Gais, 2003) that this process is facilitated by sleep, and would occur slowly over several nights.

Only one study has used the recording paradigm to test whether declarative learning is related to increases in Stage 2 sleep spindles. In this very recent study, Gais, Molle, Helms & Born (2002) found that spindle density increased following declarative learning on a paired-associates task in the first half of the night. They did not find any difference in duration of Stage 2 sleep or sigma power (12 to 14 Hz) following learning. However, they found that spindle density (spindles/30sec) was positively correlated with recall of paired-associates before and after sleep. The results from this study are inconclusive for a number of reasons. First, it is not clear how they controlled for other types of learning during the period immediately preceding sleep recording. If any simple procedural learning occurred prior to the recording night, the increase in sleep spindles could be a result of learning other than the paired-associates task. Second, there was no increase in Sigma power, indicating that the automated sleep spindle counting method (using an automatic computer algorithm) used in this study may not be reliable. Lastly, their findings are inconsistent with previous studies showing that selective Stage 2 sleep deprivation does not impair declarative memory performance on a paired-associates task. Taken together, these findings need replication controlling for additional learning comparison groups, and visual scoring of sleep spindles.

Focus of the present study

Two stages of sleep have been identified as being particularly important for memory consolidation which include REM sleep in humans (Hennevin, Hars, Maho & Bloch, 1995; Maquet, 2001; McGrath & Cohen, 1978; Smith, 1995), and animals (Smith, 1985; 1993; Steriade, Kitsikis & Oakson, 1979), and more recently Stage 2 sleep (Nader and Smith, 2003). The focus of this area of research has been on the relationship between REM sleep and procedural memory. In particular, improvement on tasks such as the Wff'n Proof Task (Smith & Fazekas, 1997; Smith & Weeden, 1990), the Tower of Hanoi (Conway & Smith, 1994), and the mirror tracing task (Plihal & Born, 1997) have all been linked to REM sleep. The common denominator with these memory tasks is that they all involve learning of a complex or novel rule or procedure to improve on task performance. In other words, performance on these tasks involves cognitive logic skills or cognitive translations. This subtype of memory has been termed *cognitive procedural memory* (Smith 1995; 2001). Recently, researchers have described a relationship between Stage 2 sleep and another subtype of procedural memory termed *simple procedural memory* for tasks that have very simple cognitive attributes and primarily involve implicit motor skills learning. Tasks such as the simple tracing task (Tweed, Aubrey, Nader, & Smith, 1999) and the pursuit rotor (Smith & MacNeill, 1994) are sensitive to Stage 2 sleep deprivation.

One of the defining characteristics of Stage 2 sleep is the sleep spindle. Sleep spindles result from the oscillatory firing of widespread thalamocortical neurons (Figure 3) (Steriade & Amzica, 1998). Sleep spindles have been found to be involved in the consolidation of simple procedural memory (Fogel, Jacob & Smith, 2002). In this study, it was found that motor skills learning increased the amount of Stage 2 sleep and sleep

spindles. Furthermore, preliminary findings indicated that individuals with a higher Performance IQ had a greater increase in spindle activity than those with a lower Performance IQ (Fogel, 2001), but not Verbal or Full Scale IQ. Subsequent analyses revealed that Performance IQ accounted for 72% of the inter-individual differences in IQ (Fogel & Smith, 2003). It is not yet understood where the increase in spindle activity resulting from new learning is localized, and if these spindles are qualitatively different from individuals not engaged in intense simple procedural learning.

The present study was designed to investigate changes in Stage 2 sleep and REM sleep after new learning. An attempt to integrate previous research was made (Fogel, Jacob & Smith, 2002; Nader & Smith, 2003; Tweed et al., 1999; Born, et al., 2002). Furthermore, the topographic and frequency characteristics of the learning-related changes in sleep spindles were investigated. To that end, a better understanding of the spatial and temporal aspects of the sleep spindle that are related to learning may be achieved. Three different types of memory tasks (simple procedural, cognitive procedural and declarative) have been chosen to investigate whether the changes observed with different types of learning result in increases in sleep-related brain activity. These findings will be discussed within the interpretive lens of memory consolidation theory.

Gilbert (2001) in his *outline of brain function* suggests that conscious memories are dependent on contextual input from either the hippocampus or the basal ganglia. As the connections in layer 2/3 of the cortex become stronger by way of long-term potentiation, they require less input from contextual sources such as the hippocampus and the basal ganglia. On the other hand, non-conscious memories are dependent on the cerebellum and connections with the thalamus and the cortex. The distinction between

conscious and non-conscious is now somewhat antiquated and difficult to test empirically. It is likely that this distinction is more useful only as a heuristic for describing an underlying distinction of cognitive complexity. Cognitively complex memories require greater attention and more cognitive resources that require conscious thought. By definition, conscious thought is context dependent. In other words, as cognitive complexity increases, so does the need for contextual information to encode and retrieve memory. Grafton et al, (1995) has argued that different structures are involved in learning depending on the level of awareness of the individual. This may account for the need for contextual input from structures such as the hippocampus and the basal ganglia for cognitively complex types of memory. Declarative memory is dependent on the hippocampus (Mottoghy et al., 1999) whereas cognitive motor procedural memory is dependent on the basal ganglia (Balslev et al. 2002; Grafton, Mazziotta, & Presty, 1992). Conversely, cognitively simple memories require less attention and fewer cognitive resources that do not necessarily require conscious thought (Figure 4). Simple motor procedural memory is dependent on the cerebellum (Grafton et al. 1992; Grafton, Woods, & Tyszka 1994), requires spaced and repeated stimulation for LTP to occur in the neocortex (Chapman et al., 1998; Trepel & Racine, 1998), and is dependent on thalamocortical oscillations (Steriade, Jones & Llinas, 1990).

In line with Gilbert's model, simple procedural learning should increase activity in cerebellar-thalamo-cortical units. Therefore, an increase in sleep spindles (more activity in thalamocortical units) should result from new simple procedural learning. Cognitive procedural memory requires visual input, and he speculates that REM sleep (specifically, dream activity during REM) would provide the right conditions (provide context) for the

consolidation of context dependent memory. Rapid eye movements have been found to increase following cognitive procedural learning (Smith, et al., submitted) and have been suggested to be a mechanism in the consolidation of cognitive procedural memory during sleep. Thus, the number of REMs should increase as a result of new cognitive procedural learning. Declarative memory is dependent on the hippocampus. Declarative memory requires contextual input and acetylcholine for LTP to occur. Graves, Pack and Abel (2001) suggest that the cholinergic activity in the hippocampus during REM sleep may be related to consolidation of hippocampus-dependent memory. Theta frequency activity is associated with LTP in the hippocampus (Tesche & Karhu, 1978), and predominates REM sleep (Cantero, Atienza, Stickgold, et al, 2003). Thus, it is likely that if declarative memory were consolidated during sleep, an increase in theta activity would occur during REM sleep.

It is hypothesized that following *simple* procedural learning, an increase in spindles and the duration of Stage 2 sleep will be observed over baseline. No change in the number of REMs or the duration of REM sleep is expected after simple procedural learning since it has been found that simple procedural memory is sensitive to only Stage 2 sleep deprivation (Tweed et al., 1999). Since sleep spindles continue throughout Stage 3 and 4 sleep, it is hypothesized that spindles in SWS will also increase from baseline to test night following simple procedural learning. Since sleep spindles oscillate in the 12 to 16 Hz range it is expected that sigma power during Stage 2, 3 and 4 sleep will follow the same pattern as sleep spindles and increase following simple procedural learning only. It is expected that the brain regions that are active during learning will be reactivated during sleep, and may provide additional support for task-specific memory consolidation during

sleep. For simple procedural learning, the underlying areas of the cortex such as motor cortices and possibly visual cortex may be more active following simple procedural learning relative to baseline and controls. This is based on the assumption that during learning, the refinement of eye-hand coordination would involve activation of the motor and visual cortex. Thus, it would be expected that there would be an increase in Sigma power at centro-parietal and occipital regions following new simple procedural learning.

Sleep spindles are not the only phasic event that characterizes Stage 2 sleep. K-complexes also serve to identify Stage 2 sleep. It has been suggested that the combined effect of sleep spindles and slow oscillations during NREM sleep leads to the production of k-complexes (Steriade & Amzica, 1998). It will be necessary to determine if other Stage 2 electrophysiological events such as k-complexes fluctuate with new learning to rule out the possibility that Stage 2 sleep changes globally across multiple indices. There is a controversy about the function of the k-complex. Some believe it is a sleep protective mechanism (inhibitory), while others believe it is an arousal (for review, see: Campbell, Bell & Bastien, 1992). Thus, if k-complexes remain constant following new learning, whereas sleep spindles change, it would suggest that sleep spindles were specific to memory consolidation and not simply a marker of increased Stage 2 sleep phenomena. K-complexes have been proposed to be related to sleep maintenance and information processing during sleep (Oswald, Taylor & Treisman, 1960; Salisbury & Squires, 1993; for review see Bastien, Crowley & Colrain, 2002). It was hypothesized that k-complexes will not increase following new learning since it was believed that spindle-dependent memory consolidation might be dissociable from k-complex-dependent processing of salient or familiar stimuli.

Following *cognitive* procedural learning, it is hypothesized that there will be an increase in the number of REMs and/or REM density. No change was expected in sleep spindles or Stage 2 sleep as a result of cognitive procedural learning since cognitive procedural memory has been found to be sensitive to REM sleep deprivation only (Tweed et al., 1999).

The formation of declarative memory has been found to be related to theta activity, whereby theta is suggested as the frequency that the hippocampus communicates with the other structures (Klimesch, 1996), and is involved in the induction of LTP (Larson, Wong, & Lynch, 1986). It was hypothesized that there would be an increase in theta activity during REM sleep following declarative learning.

Based on previous findings (Jobert, et al., 1992), it can be expected that fast spindles will be localized to posterior regions, and slow spindles to anterior regions. These distinct spindles may be differentially associated with learning. Based on Gilbert's model, slower frequency activity is associated with more efficient LTP, whereas faster frequencies are associated with less efficient LTP. Based on these findings, it was expected that there would be an increase in spindles in the lower frequencies (12 to 14 Hz) after new simple procedural learning.

It was also expected that Performance IQ, but not Verbal or Full-Scale IQ would be positively related to baseline spindle activity. This prediction is in line with Gilbert's suggestion that the greater the number of inputs to the layer 2/3 cortical neurons, the greater the capacity for memorizing units to function with greater efficiency. Based on this, it is expected that a greater number of baseline spindles reflects a healthier thalamocortical system, one that has greater potential for learning new behaviours,

especially involving motor skills. Thus, Performance IQ would provide a similar index of this greater capacity for learning, and it is expected to be correlated with the number of baseline sleep spindles. In addition, it would also be expected that Performance IQ would be positively correlated with the increase in spindles after new learning.

Method

Participants

Participants were recruited primarily from the introductory psychology course at Brock University from the ages of 18 to 26. Advertisements for the study were posted on the web-based participant recruitment site, whereby participants could view postings for a variety of research studies at Brock University. Posters hung along the corridors of Brock University were also used to recruit participants. An initial telephone screening interview was used to exclude participants for left-handedness, unusual sleep patterns (outside of 11:00PM to 7:00AM), shift work, head injury, cigarette smoking, and chronic pain. In addition, participants were excluded for activities that were thought to confound the type of intensive learning they were engaged in during the study. Activities that involved the development of simple motor skills (for example; dance lessons or other sports activities), and complex motor skills (for example; piano lessons, video games, and strategy games such as chess). If participants were engaged in these activities regularly (more than once per week for a period of several hours), they were excluded from participating in the experiment. If they occasionally engaged in these activities (less than once per week for a period of several hours), they were asked to restrict themselves from engaging in these activities for the duration of their participation in the study. It was expected that all

participants would be engaged in *regular* amounts declarative learning since they were recruited from a university population. However, participants were screened for *above* normal levels of declarative learning (for example; studying for midterm examinations, or writing term papers). If participants were preparing for exams or writing papers in the week immediately preceding or during overnight testing and recording sessions the participants were not scheduled to participate at that time. Participants were subsequently screened using a sleep-wake questionnaire (Yoshitake, 1978) to screen candidates with symptoms of sleep disorders, unusual sleep patterns or excessive daytime sleepiness (Appendix A). The Horne & Osterberg (1976) Circadian Rhythm Questionnaire (Appendix B) was administered to screen candidates who were extreme morning or evening types. Participants were asked to avoid the use of mobile phones on days preceding overnight EEG recording. It has been found that exposure to electromagnetic radiation similar to that of cellular phones increased brain activity in the spindle frequency range during sleep (Huber, Graf, Cote, et al., 2000). If participants reported using cell phones during the period preceding EEG recording nights, they were excluded from further study. No participants were excluded for this reason. All participants spent three uninterrupted consecutive nights in the Brock University Sleep Research Laboratory, including acclimatization/screening, baseline, and test night. The first overnight recording session served as an acclimatization night and to screen candidates for poor sleep quality, the appearance of periodic limb movements, or respiratory events. Seventeen participants were excluded based on the telephone interview criteria, and three dropped out after the first screening night. Two participants were excluded following the clinical screening night due to the appearance of periodic limb movements associated

with regular arousals throughout the night. One participant was excluded from all analyses based on several factors including not following instructions during the experiment, alpha intrusion on the EEG throughout both baseline and test nights, and poor sleep quality due to repeated awakenings throughout the night. Her data was replaced by pilot data to maintain the sample size. The pilot data set was complete with the exception that data was missing on the re-test for the Pursuit Rotor due to a computer malfunction that resulted in the data file not being generated following the task, and missing data on the IQ measures due to the participant refusing to have their IQ measured from inadequate confidentiality.

The final sample size included thirty females and six males aged 18 to 26 (M = 20.28, SD = 5.26) who spent three consecutive nights in the sleep laboratory. Sample size was based on power estimates from previous data (Fogel, Jacob & Smith, 2002). Participants were randomly assigned to one of the four experimental groups including (nine participants in each group): (1) cognitive procedural memory task (C-PM); (2) simple procedural memory task (S-PM); (3) declarative memory task (DM); or (4) control (C).

Materials

Screening Questionnaires and Sleep Log. A telephone screening interview (Appendix C) was used to measure self-reported behaviours in three domains including sleep-related, learning-related, and health-related areas. The sleep-related questions enquired about unusual sleep patterns, tiredness, sleep maintenance difficulties and shift work. The health-related domain addressed head injury, depression, medications, cigarette smoking, and chronic pain. The learning-related domain addressed handedness,

and the frequency and type of learning that participants normally engaged. If participants qualified all of the criteria outlined in the telephone interview, informed consent was obtained (Appendix D), and additional screening questionnaires were used in an orientation/screening session to collect more information on sleep quality and quantity. This package included the Horne and Osterberg (1976) Circadian Rhythm Questionnaire that was used to screen participants for extreme morningness or extreme eveningness. The Circadian Rhythm Questionnaire measures self-report judgment of peak performance times in sleep behaviour, physical tiredness, and alertness. The 19-item scale consists of five visual-analogue scales and 14 four-point Likert scales. Participants were considered extreme on morningness or eveningness if they responded at either extreme on more than 10 of the items. The Yoshitake (1978) Fatigue Questionnaire was used to screen candidates for daytime fatigue. The Fatigue Questionnaire measures physical and mental symptoms of fatigue such as "I feel unable to stand up straight" and "I am unable to concentrate" in a 30-item forced choice format (ie; yes or no). A sleep-wake questionnaire (Appendix E) was used to screen candidates for sleep quality, intake of drugs and alcohol, chronic pain, symptoms of sleep disorders, daytime tiredness, shift work, prescription and non-prescription medications, family sleep history, and health-related problems (ie; back pain, diabetes, and head injury). If participants met the criteria for inclusion in the rest of the study, they were given a sleep and activity diary (Appendix F) that measured sleep and wake times, caffeine and alcohol consumption, type and duration of physical exercise, studying, and other leisure activities. The activities measured with this instrument were assessed daily until the completion of the last overnight spent in the sleep laboratory.

Intelligence tests. The Multidimensional Aptitude Battery II (MAB-II) was used to measure general intelligence (Jackson, 1998). The MAB-II is a paper and pencil test that takes between 60 and 90 minutes to complete. Scoring the MAB-II is straightforward with the use of answer keys, formulae and step-by-step instructions included in the MAB-II kit (Sigma Assessment Systems). IQ scores and subscales can be computed reliably and objectively since no judgments on performance or behaviour are required by the administrator of the test. The MAB-II yields three IQ scales including Verbal, Performance, and a composite of the two (Full Scale IQ). Verbal IQ is composed of five subscales including information, comprehension, arithmetic, similarities, and vocabulary. Performance IQ is also composed of five subscales including digit symbol, picture comprehension, spatial, picture arrangement, and object assembly.

Learning tasks. The mirror-tracing task has been characterized as being a non-verbal procedural task that involves cognitive translations and implicit learning (Smith, 1995). The task involves tracing around 14 figures (Appendix G) with a pen as quickly and accurately as possible. Two concentric lines 5 mm apart outline the figures. The goal of the task is to trace around the figure, keeping between the lines without touching them. The participant must do this by watching their hand in a mirror. A shade blocks the participant from directly tracking their hand movements. The dependent measure for this task is the number of times the participant touches the figure outlines.

The pursuit rotor involves gross controlled motor movements, rhythm and tracking. It is a cognitively simple task, with a set of movements that are not unusual. Improvement in this task occurs as a result of refinement of motor skills by implicit learning. This task required the participant to follow a rotating target in a circular track

using a hand-held computer mouse pointing device. A computerized version of the pursuit rotor was used as it has been found to produce equivalent results to the pursuit rotor apparatus (Fillmore, 2003). Participants completed forty sets of 30-second trials at 30 RPM (for a total of 15 rotations per trial, or 600 rotations in total) with 60-second rest intervals between sets of trials. The target revolved around the track at 30 revolutions per minute. Thirty RPM was chosen as opposed to a faster speed because the strategy used to improve on the task at slower speeds is different than the strategy used to improve at the task at higher speeds (60 RPM) (Siegel, 1990). Siegel found that at 30 RPM, individuals make less hits but are able to track the target for longer durations and have a greater increase on subsequent trials in time on target than at 60 RPM. At 60 RPM, the task is more challenging, but individuals make more hits and have less time on target than at 30 RPM. This implies that faster speeds are detrimental to developing a pattern of rhythm in tracking. Percent time on target was counted when contact was made between the rotating target and the crosshairs of the computer pointer. A 440Hz, 60DB tone sounded when the stylus was on target. This tone served as positive feedback for task performance.

The paired-associates task was chosen because it has been previously found to be related to both Stage 2 sleep (Gais et al., 2002) and REM sleep (Barrett & Ekstrand, 1972). It is both verbal and cognitive in nature and involves explicit declarative learning. The testing procedures, number of trials, and number of word pairs, used by Gais, et al. (2002) were used in this experiment. Words were selected for high concreteness, low emotionality, and word length (5 to 11 letters). Participants learned 168 word pairs presented in individual pairs during acquisition. This procedure was repeated twice, once for 106 seconds and again for 70 seconds. Participants were instructed to visually relate

the words to one another with mental imagery to try to memorize the pairs. Recall was tested immediately after learning. During testing, the first word in the pair of study words was presented alone. The participant was instructed to respond with the other member of the pair from memory. Responses were typed and displayed on the computer screen next to the first word in the pair. Participants could correct themselves if they wished, and could begin the next word-pair trial by pressing the "Enter" key on the computer keyboard.

Polysomnographic Recording Apparatus. EEG, EOG, and EMG were recorded using a 64-channel Mizar SD32+ digital amplifier and Sandman™ and Spyder™ software (Tyco™). EEG was recorded at 256Hz with hardware filters set to cut off frequencies below 0.099 and above 115.2 Hz (high frequency cut off = 0.45 x sampling rate). Polysomnographic digital data was stored on a hard drive using a Pentium-class personal computer. Gold-plated pure sliver Grass™ electrodes were used for all electrophysiological recordings using a water-soluble Ten20™ conductive paste with the proper resistive and adhesive properties for accurate recordings. Electrodes were held in place overnight with the use of either cotton gauze pads or 3M™ Micropore™ surgical tape. Respiratory effort was measured using adjustable elastic respiratory effort belts (Braebon Inc.).

Procedure

Table 1 outlines the experimental design. Participants were administered the MAB-II IQ test one week prior to any in-laboratory overnight testing and recording. During the interim week, they were asked to keep a log of their sleep-wake cycle, eating, drinking and exercise patterns using the Sleep Research Laboratory's sleep journal to

further screen for any unusual sleeping or confounding daily activities.

Sleep quality (greater number of arousals and poorer sleep efficiency) and quantity (longer sleep onset) is typically different on the first night in the sleep laboratory (Williams, 1966). The acclimatization night served to control for the "first night effect" (Williams, 1966) and to allow the participants to become comfortable and accustomed to sleeping in the sleep laboratory. In addition, the acclimatization night served as a screening night to exclude participants from further involvement in the study due to any sleep related disorders including sleep apnea and periodic leg movements. The baseline night was used to collect baseline EEG for within subject comparisons to the test night. The week before overnight recordings participants were instructed to keep regular sleep and wake times, avoid excessive drinking and exercise. Before each overnight recording session, participants were asked to awake by 8:00 AM that morning, avoid napping, have no more than one caffeinated beverage in the morning, and none thereafter, avoid any alcoholic beverages, and to avoid exercise. On the test night, at 2100 hrs participants were asked to perform one of the following memory tasks, including the mirror tracing task, pursuit rotor, paired-associates, or no learning task (control), prior to sleep onset. One week thereafter, they repeated the practice session at 2100 hrs. For participating in the study they received a 3-hour course credit in an introductory psychology course (if enrolled) and paid $25.00 per sleep night. At the end of their participation in the study, participants received written feedback regarding (Appendix H) the purpose of the study. No individual data or feedback about their sleep quality or performance on tasks or questionnaires was given out to the participants. Study procedures were approved by the Brock University Research Ethics Board in accordance with Tri-council guidelines and

regulations on ethical conduct for research involving humans.

Intelligence Testing. The MAB-II was used to assess IQ based on its ease of administration (pen and paper format) and high correlations (.91) with the WAIS (Jackson, 1998). Participant's IQs were tested at 1200 to 1330 hours, one week prior to any in-laboratory testing following the procedures outlined in the MAB-II IQ test manual (Jackson, 1998).

Memory Tasks. Three different memory tasks were used in separate experimental conditions. The training sessions for all groups began at 2100 hrs, followed by the test night in the laboratory starting at 2300 hrs. One week following the first training session, the same task was repeated to assess performance improvements during the retention interval. The cognitive procedural memory (C-PM) group performed the Mirror tracing task that involves implicit learning for complex cognitive procedural translations. The simple procedural memory (S-PM) group performed the pursuit rotor task that involves very simple procedural motor skills and eye-hand coordination. Lastly, the declarative memory (DM) group performed the paired-associates task, a verbal and declarative task involving explicit learning. The control group spent the equivalent amount of time filling out sleep-related questionnaires and forms to collect demographic information.

Physiological Recording Parameters. EEG was recorded from: Fp1, Fp2, F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, Oz, and O2 according to the international 10-20 system for electrode placement and was referenced to Fpz and grounded to AFz. EEG channels were software filtered to cut off frequencies below 0.5Hz and above 35 Hz. A 35Hz (high frequency cut off) software filter was applied to the EOG channels, and a 0.1Hz (low frequency cut off) filter was applied to the EMG. A Notch (60Hz) filter was applied to all

channels to eliminate electrical noise.

On the acclimatization night, EEG was recorded from all 14 active sites listed above and two electrodes were placed on each leg to record EMG to screen participants for periodic leg movements. In addition, respiratory bands around the abdomen and chest were used to screen participants for sleep apnea. EOG was recorded from each eye on the lateral side of the orbit and on the right superior side of the orbit for sleep stage identification.

The baseline and test night sleep recordings were scored using standard criteria in 30-second epochs (Rechtschaffen & Kales, 1968) with one exception involving the scoring of REM sleep. According to Rechtschaffen and Kales, "low amplitude EMG contributes little to the scoring of sleep stages, but the presence of 'relatively elevated' tonic EMG… (precludes) the scoring of stage REM." In addition, "there is an absolute absence of sleep spindles and k-complexes", and one should "score the record up to the last sleep spindle or k-complex as Stage 2 irrespective of EMG level." In this data, at transition points, sometimes k-complexes and spindles persisted in the low amplitude EEG, with relatively low tonic EMG and clear phasic eye movements. In this case, these epochs were scored as REM.

Sleep spindles were visually counted from Cz with the additional aid of a filtered channel that displayed only 12 to 16 Hz activity. Each sleep spindle was also measured in duration (seconds). Sleep spindles were counted and measured in Stage 2, 3 and 4 sleep separately across the entire night. All spindles included in the count exceeded 0.5sec, and had typical fusiform (waxing and waning amplitude) spindle morphology. This usually meant that their maximum amplitude also exceeded 10uV. However, there was no

minimum amplitude criteria set in accordance with standard sleep scoring procedures (Rechtschaffen & Kales, 1968).

K-complexes, sleep spindles and rapid eye movements were all scored blindly. Recoding was done by an independent person, who kept the code key in their possession until scoring was completed. Each sleep record was recoded and identified by a randomly assigned number. Thus, there was no identifiable information about the participant, recording night, or learning condition for all sleep records scored. In order to have a more inclusive count of k-complexes, a separate scoring scheme was used to count k-complexes that adhered strictly to standard criteria (Rechtschaffen & Kales, 1968). K-complexes were visually counted from Fz, Cz and Pz with the aid of a filtered 12 to 16 Hz channel to identify k-complexes that occurred at the same time as spindles. K-complexes were counted if they exceeded 75uV, had both a negative and positive deflection, and were maximal at frontal sites. Three categories of k-complexes were identified: the total number of k-complexes, k-complexes that occurred with spindles (spindle activity that occurred prior to, overlapping, or following the k-complex), and k-complexes that occurred in the absence of spindles. Initially, k-arousals (k-complexes that occurred immediately prior to an arousal in the EEG) were considered as a category, however, so few k–arousals were identified, that they were eliminated from further analysis. K-complexes were not counted in Stage 3 or 4 sleep as it is too difficult to discriminate between spontaneous k-complexes and delta waves. Rapid eye movements were visually scored from left and right eye channels re-referenced offline to an average of A1 and A2. EOG channels were filtered at 0.5Hz to reduce slow drift on these channels. This filtering technique aided in the identification of rapid eye movements, and

eliminated only very slow eye movements. Any conjugal eye movements that exceeded 25uV were included in the count (Smith & Smith, 2003). All phasic activity including sleep spindles, k-complexes and rapid eye movements were scored in the absence of movement artifact (contamination of EMG activity onto the EEG channels).

Power spectral analysis of the EEG was done using a Fast Fourier Transformation (FFT) in each sleep stage separately including Stage 2, 3, 4 and REM sleep. Stage 2 sleep was analyzed in two separate halves to determine if time of night was an important factor for learning dependent changes in sleep (Barret & Ekstrand, 1972; Ekstrand, Barrett, Weat & Meier, 1977; Fowler et al., 1973; Plihal & Born, 1997; Yaroush et al., 1971). The duration of the night from sleep onset to lights on was divided to mark the midpoint of the night for each participant and each night separately. All Stage 2 from the first half and second half of the night were analyzed separately. For all FFT analyses, the EEG was analyzed in 2sec Hanning windows with a 75% overlap, and was re-referenced to an average of A1 and A2. Low frequency EEG was filtered at 0.5 Hz using a software filter, and high frequency EEG cut offs remained at the hardware setting of 115.2Hz described above. The FFT was binned into eight frequency bins including delta (.5 - 4Hz), theta (4 – 8Hz), low alpha (8 – 10Hz), high alpha (10 – 12Hz), low sigma (12 – 14Hz), high sigma (14 – 16Hz), beta (16 – 35Hz) and gamma (35 – 60Hz). Any epoch with artifact from EMG, EKG or EEG clipping was excluded from the analysis. All FFT data was Log10 transformed since raw power is positively skewed. This transformation is used to normalize the distribution of scores.

Data Analyses

Learning Data. Paired t-tests were used to assess the hypothesized improvements

in performance from training to re-testing one week later on the memory tasks in each learning group separately. The percentage of time spent on target, the number of tracing errors, and the number of correctly recalled word pairs were analyzed in the S-PM condition, C-PM group and the DM group, respectively.

IQ Data. Because of the inter-individual differences in sleep spindles (Silverstein & Levy, 1975), Performance IQ was considered an ideal covariate to partial out the inter-individual differences in sleep spindles (Fogel & Smith, 2003) that may have confounded the repeated measures design used in this experiment. Simple bivariate Pearson's correlations coefficients were used to determine the suitability of Performance IQ as a covariate for subsequent analyses. If significant correlations existed between Performance IQ and baseline sleep spindles (spindle number, density, or duration), then a test of the assumption of parallel slopes was conducted to determine if IQ could be used as a covariate. Violation of this assumption would have indicated that the relationship between the dependent variable (spindle number, density or duration) and the covariate was not the same across levels of the independent variable (learning condition). To test the assumption of parallel slopes, a multiple regression with spindles as the dependent variable, with Performance IQ and learning condition (dummy coded) was entered on the first step, followed by the Performance IQ by group interaction term on the second step. The test of the assumption was assessed at step 2 of the model. If the interaction of Performance IQ by group accounted for a significant proportion of variability in spindles, then it could be concluded that the regression lines for each group were not parallel, and the assumption of parallel slopes had been violated. If there were no significant correlations between spindles and IQ, then IQ was not considered as a suitable covariate

for subsequent analyses. The baseline number of rapid eye movements was also correlated with Performance, Verbal, and Full Scale IQ to determine if other phasic markers of sleep-dependent memory consolidation were related to IQ. Previous research has shown that the relationship between IQ and sleep spindles was most robust in individuals with above average IQ scores (Fogel, 2001). To determine if Performance IQ was related to baseline spindle measures, participants were categorized into 3 groups divided by the 33rd percentile and 66th percentile. In this data set, this yielded a low IQ group (N = 12), with Performance IQ scores from 77 to 107, a medium IQ group (N = 12) with Performance IQ scores from 108 to 114, and a high IQ group (N = 11) with Performance IQ scores that ranged from 115 to 126. For comparison, Verbal IQ, and Full Scale IQ were split into three groups at the 33rd percentile and 66th percentile. This yielded a low group (N = 14) with Verbal IQ scores that ranged from 76 to 108, a medium Verbal IQ group (N = 10) with scores that ranged from 109 to 113, and a high Verbal IQ group (N = 11) with scores that ranged from 114 to 128. Full Scale IQ was also split into a low IQ group (N = 12) with scores that ranged from 75 to 110, a medium IQ group (N = 14) with scores that ranged from 111 to 115, and a high IQ group (N = 9) with scores that ranged from 116 to 127.

Sleep Data. All analyses were carried out using the protected tests strategy outlined by Howell (2002). The goal of this method is to provide a systematic method for investigating important sources of variance while controlling for family-wise error rates. Using this method, more complex overall analyses are followed up by simple effects analyses and subsequent post hoc or planned comparisons. The alpha rate at each subsequent analysis is said to be "protected" by the preceding analysis since follow-up

procedures are only conducted if preceding analyses are statistically significant. In the sleep architecture data set, 2 x 4 (night x learning group) mixed-design ANOVAs were used to analyze differences in minutes for each of Stage 1, 2, 3, 4 and REM separately. If a significant group by night interaction was detected, a follow-up simple effect one-way ANOVA on baseline night data only was used to determine if groups differed significantly at baseline. If it was found that groups differed at baseline, a one-way ANCOVA was used to partial out baseline differences to determine which groups differed on test night. If a significant main effect for learning group was found using the one-way ANCOVA, independent t-tests with a Bonferroni correction to control for comparison-wise error rates were used. If groups did not differ at baseline, paired Bonferroni t-tests using the pooled error term from the overall interaction were used to test changes from baseline to test night in each group. The use of paired Bonferroni t–tests using a pooled error term is also recommended by Howell (2002, chapter 14) for follow-up tests on within subjects effects when the underlying assumptions for the overall ANOVA have been met. The assumptions of sphericity and homogeneity of variance were examined for each analysis carried out using this strategy. Whenever an assumption was violated, the steps for correcting the problem were described.

Due to the highly variable durations of Stage 3 and 4 sleep across individuals, Stage 3 and 4 sleep were collapsed into slow wave sleep (SWS). This was done for sleep scoring, spindle counts, and FFT analyses.

Sleep Spindles, K-complexes and Rapid Eye Movements. A mixed design 2 x 4 (night x learning group) ANOVA was used to analyze differences in Stage 2 and SWS sleep spindles. The same analytic strategy was used for sleep spindles as described above

for sleep stages. The total number of sleep spindles cannot be considered as a useful measure since there was an increase in the duration of Stage 2 sleep, and it would be expected that the number of sleep spindles could increase simply based on the increase in Stage 2 sleep. Thus, the different measures of sleep spindles have been weighted by the number of minutes of Stage 2 sleep to yield a measure of spindle "density" (number of spindles per minute). Spindle duration was also measured and has been weighted in the same way (by the number of minutes of Stage 2 sleep). The same analytic strategy was used to analyze the data for spindle density and duration during SWS. A 2 x 4 (night x learning group) ANOVA was used to analyse spindle density and duration during SWS from baseline to test night for the four learning conditions.

The same analysis strategy that was outlined above for sleep spindles will be adopted for the analysis of the k-complex measures. The total density of k-complexes includes k-complexes that occur concurrently with spindles and k-complexes that occur without spindles, weighted by the number of minutes of Stage 2 sleep. A 2 x 4 (night x learning group) ANOVA was used to determine if the total number of k-complexes during Stage 2 sleep changed following learning. k-complexes that occurred in the absence of spindles, and k-complexes that occurred in the presence of spindles were analyzed using the same strategy.

Regardless of whether or not there was a significant change in the duration of REM sleep, the changes that were observed in the duration of Stage 2 sleep might be expected to have an effect on the other sleep stages in ways that were not statistically robust. Thus, the number of rapid eye movements per minute (REM density) were analyzed as opposed to the total number of rapid eye movements. The same data analysis

strategy described for the sleep spindle data was used to analyze REM density.

Spectral Analysis of Sleep Using FFT. Due to the amount of data that has been collected here (seventy-two 8-hour nights from 14 EEG sites, in five sleep stages, and 8 frequency bands), a hypothesis-driven analytic strategy was adopted here to avoid inflating Type-I error. The FFT data were analyzed using the same series of statistical tests that were described for the sleep stage data in the previous section, and protected test strategy outlined by Howell (2002). Additional safeguards were used for the FFT data set, namely the statistical "top down" approach described below.

Since the Stage 2 data was analyzed using FFT in such a way that the first half and second half of the night could be analyzed separately, and the sigma band was spilt into low (12 – 14Hz) and high (14 – 16Hz) sigma power as well as total sigma (12 – 16Hz), a "top-down" approach was used to analyze these data. First, total sigma for Stage 2 sleep for the whole night was analyzed at midline sites (Fz, Cz, Pz, Oz) to determine if there were any changes in the total sigma band over the course of the entire night. If total sigma power changed from baseline to test night as a function of learning condition, the other power bands (delta, theta, low alpha, high alpha, beta and gamma) were tested to determine if the changes in power during Stage 2 sleep were specific to the sigma band, or changed globally across all bands. This was done only at the site where the effect was found to be maximal. If the hypothesized differences were statistically significant, total sigma power for the first and second halves of the night was analyzed separately at the site where the effect was maximal. If changes in the total sigma band varied as a function of learning condition in a particular part of the night, then the sigma band was analyzed separately as low and high sigma to determine if fast or slow sigma power was of

particular importance to sleep-dependent memory consolidation at the site where the effect was maximal. This strategy was designed to reduce family-wise error rates to protect against the inflation of Type-I error. The logic for this strategy is based on the fact that subsequent analyses were only carried out if the hypothesized differences existed at the preceding step. Thus, the same sources of variability were tested using increasingly distinctive between groups factors, thus the Type-I error was protected by the overall analyses that preceded subsequent analyses. The same analytic strategy was used to investigate learning-related changes in spectral power during SWS and REM sleep with the exception that SWS and REM sleep were not categorized into the first and second halves of the night. This was due to inadequate amounts of SWS data in the second half of the night and REM in the first half of the night. Theta during REM sleep was analyzed at midline sites (Fz, Cz, Pz, Oz). If theta power changed from baseline to test night as a function of learning condition, the other power bands (delta, alpha, total sigma, beta and gamma) were tested to determine if the changes in power during REM sleep were specific to the theta band, or changed globally across all bands. This was done at the site where the effect is found to be maximal. Follow-up procedures were the same as outlined for Stage 2 sleep and SWS power. The mean amount of data submitted for FFT in each stage of sleep and learning condition are listed in Table 2.

There was no EEG data from the Pz site for 2 participants on the baseline night due to irresolvable signal problem with that channel. For these data, the power from the four nearest neighbors (Cz, P3, P4, Oz) was averaged and included in the data set. Two participants were excluded from all FFT data analyses due to poor impedances (> 100 KOhm) on the recording reference electrodes (Fpz). In addition, two more participants

were excluded based on unusually high power across all bands. This problem occurred only after re-referencing the active sites to an average of A1 and A2, so the unusually high power was attributed to poor recording of A1 and A2. After the removal of these participants, there were n = 8 in the S-PM group, n = 7 in the C-PM group, n = 9 in the DM group and n = 8 in the C group. In the following section, the analysis strategy for investigating the topography of this effect is discussed in greater detail to describe the extent of the effect across the scalp.

Topography of the Learning-dependent Changes in Spectral Power. The main findings from the spectral analysis of sleep using FFT described in the previous section were investigated further to determine how the changes in spectral power during sleep from learning were distributed across the scalp. The previous analyses have established where the statistical effects were maximal along the midline of the scalp. For the following analyses, a less conservative approach was taken in order to gain a more descriptive picture of the topography of these effects. In the groups that were found to have a change in power from baseline to test night, in addition to reporting statistically significant results ($p < .05$), results from paired t-tests at a less conservative p-level ($p < .10$) were reported to determine how the effect was distributed across the scalp. Note that gains in descriptive detail are at the expense of inflating the probability of Type-I error. However, these tests have been carried out only following analyses that employed a much more conservative statistical approach. Topographic maps for each participant will be used to help interpret the findings from the paired t-tests conducted at all sites.

Results

Learning Data

It was predicted that each learning group would have a significant gain in performance after a one-week retention interval filled with no practice. Paired t-tests were used to test pre-post changes in performance in each memory group. It was found that all groups improved on performance from test to re-test (Table 3). The C-PM group made fewer errors on the mirror tracing task after the retention interval ($t(8) = 10.0$ $p < .001$) and the DM group recalled more word pairs after the retention interval ($t(8) = 4.19$, $p = .003$). The S-PM group had a higher percent time-on-target on the pursuit rotor after the retention interval ($t(7) = 4.62$, $p = .002$).

IQ Data

Upon inspection of the histograms and boxplots of each IQ scale, it was clear that the distribution of Performance IQ, Verbal IQ and Full Scale IQ scores were negatively skewed. The Kolmogorov-Smirnov Z test was used to determine if these distributions violated the assumption of normality. Performance IQ ($Z(35) = 0.180$, $p = .006$), Verbal IQ ($Z(35) = 0.214$, $p < .001$) and Full Scale IQ ($Z(35) = 0.222$, $p < .001$) deviated significantly from a normal distribution. Log10 transformations were used to normalize the distributions (Tabachnick & Fidell, 2001). First, the distributions were reflected. This was accomplished by adding 1 to the value of the highest score (separately for each IQ scale) and subtracting each score from that constant. The data is then transformed by calculating the Log10 of each score. Following the transformations, from the inspection of histograms and boxplots the shape of the distributions did improve. However, the Kolmogorov-Smirnov Z test revealed that the distribution of scores remained

significantly different from a normal distribution (Performance IQ ($Z(35) = 0.148$, $p = .049$, Verbal IQ ($Z(35) = 0.223$, $p < .001$) and Full Scale IQ ($Z(35) = 0.160$, $p = .023$)), thus the original raw IQ scores were used for correlational analyses.

As predicted, there was a significant positive correlation between the change in the number of sleep spindles after learning and Performance IQ ($r(33) = .36$, $p = .032$) and Full Scale IQ ($r(33) = .34$, $p = .044$), but not Verbal IQ ($r(33) = .25$, $p = .155$).

Contrary to previous findings, no relationship between Performance IQ and baseline sleep spindles (number of spindles, density or duration) was found. When split into low, medium and high IQ, there were no significant correlations between baseline spindle measures and IQ scores in the low or medium Verbal, Performance or Full Scale IQ groups. However, there was a significant correlation between the baseline number of spindles ($r(9) = .75$, $p = .01$) and spindle density ($r(9) = .73$, $p = .01$) in the high Performance IQ group (Figure 5). Upon inspection of the scatterplots of these data, it was clear that there was one score that was outlying from the rest of the scores, and was driving the direction and magnitude of this relationship. When removed, the relationship between Performance IQ and was no longer statistically significant for baseline spindles ($r(8) = .50$, $p = .14$) or spindle density ($r(8) = .50$, $p = .14$). Despite the fact that the outlier, and thus the direction and magnitude of the relationship between Performance IQ and spindles was as hypothesized, it was decided that Performance IQ would not be an ideal covariate for subsequent analyses due to an inadequate amount of variability accounted for by the high IQ group with the outlier removed. In addition, there was no relationship between Performance IQ and spindles in the low and medium IQ groups.

Surprisingly, significant correlations were found between Verbal IQ and the

baseline number of rapid eye movements ($r(9) = .65$, $p = .029$) (Figure 6). No significant relationship was found between rapid eye movements and Performance IQ or Full Scale IQ. Table 4 displays the correlations between Performance, Verbal and Full Scale IQ and the Stage 2 spindle and REM measures.

Sleep Data

To determine if sleep architecture was affected by new learning, the duration of time spent in each stage of sleep (Stage 1, 2, SWS & REM) prior to and following learning were compared in the four learning conditions. No significant night by group interaction was found for the duration of Stage 1 sleep ($F(3, 32) = 1.46$, $p = .25$). As predicted, there was a significant night by group interaction for the duration of Stage 2 sleep ($F(3, 32) = 4.34$, $p = .01$). A one-way ANOVA was used to determine if groups had significantly different amounts of Stage 2 sleep at baseline. This analysis revealed that the groups had significantly different duration of Stage 2 sleep on the baseline night ($F(3, 32) = 3.09$, $p = .04$). A one-way ANCOVA was used to partial out baseline differences in Stage 2 sleep from test night Stage 2 sleep. This analysis revealed a significant effect of learning on test night after baseline differences have been removed ($F(3, 31) = 5.93$, $p = .003$). Independent t-tests with a Bonferroni correction were used to follow-up group differences on the duration of test night Stage 2 sleep. It was found that the S-PM group had more Stage 2 sleep on the test night than the control group ($t(8) = 3.83$, $p = .004$). The C-PM and the DM group did not differ from controls in the duration of Stage 2 sleep on test night. No significant night by group interaction was found for the duration of SWS ($F(3, 32) = 0.53$, $p = .67$) or REM sleep ($F(3, 32) = 1.25$, $p = .31$). Group means for baseline and test night are presented in Table 5.

Sleep Spindles

As predicted, a 2 x 4 (night x learning group) ANOVA revealed a significant interaction between night and learning group ($F(3, 32) = 3.02$, $p = .04$) for sleep spindle density during Stage 2 sleep (Figure 7). A one-way ANOVA was used to determine if spindle density differed significantly between groups at baseline. This analysis revealed that the groups did not have significantly different spindle densities during Stage 2 sleep on the baseline night ($F(3, 32) = 3.09$, $p = .04$). Pre-post learning differences in spindle density were compared using Bonferroni t-test using the pooled error term from the overall 2 x 4 (night x learning group) ANOVA. These tests revealed that the S-PM group had an increase of 0.89 spindles per minute from baseline ($M = 6.36$, $SD = 2.08$) to test night ($M = 7.25$, $SD = 2.09$) ($t(32) = 3.14$, $p < .05$). Spindle density did not change from baseline to test night for the DM, C-PM or control groups.

A similar pattern of results was found for Stage 2 sleep spindle duration (Figure 8). A 2 x 4 (night x learning group) ANOVA revealed a significant night by group interaction ($F(3, 32) = 5.65$, $p = .003$) for Stage 2 sleep spindle duration. A one-way ANOVA revealed that the groups did not differ significantly at baseline ($F(3, 32) = 1.35$, $p = .28$). Bonferroni t-tests were used to test pre-post differences in spindle duration. It was found that spindle duration increased from baseline ($M = 1.42$, $SD = 0.18$) to test night ($M = 1.68$, $SD = 0.12$) following S-PM learning ($t(32) = 4.21$, $p < .01$). The C-PM, DM and control groups spindle duration did not change from baseline to test night.

Upon inspection of the data, one extreme outlier for spindle density during SWS was found in the control group that produced a highly skewed distribution. There was no interaction between night and learning condition for SWS spindle density. However, this

analysis revealed a similar pattern of results as spindle density in Stage 2 sleep and the interaction did approach significance ($F(3, 32) = 2.52$, $p = .075$). It was believed that the outlier may have affected the robustness of the overall analysis, thus follow-up analyses (with the outlier included) were conducted to determine if the hypothesized increase in spindle density for the S-PM group in SWS did exist. A one-way ANOVA was run on baseline SWS spindle density, which revealed that the groups did not differ significantly at baseline ($F(3, 32) = .528$, $p = .67$). The Bonferroni t-test was used to test pre-post differences in SWS spindle density following learning. As with the Stage 2 sleep data, there was a significant increase from baseline ($M = 7.68$, $SD = 3.00$) to test night ($M = 8.75$, $SD = 2.40$) in sleep spindle density following S-PM learning during SWS ($t(32) = 3.01$, $p < .05$) but not in the C-PM, DM or control groups. A similar pattern of results was found for spindle duration in SWS. A 2 X 4 (night x Learning Group) ANOVA revealed a significant interaction between night and learning group for spindle duration ($F(3, 32) = 3.86$, $p = .018$). A one-way ANOVA on baseline SWS spindle duration revealed that the four groups did not differ on baseline SWS spindle duration ($F(3, 32) = .42$, $p = .74$). However, post-hoc t-tests did not detect any significant change in spindle duration in the S-PM, C-PM, DM or C groups.

K-Complexes

There was no change from baseline to test night in the total number of k-complexes as a function of learning condition. It is worth noting that the assumption of homogeneity of variance was violated for night 2 only. Further, the between-within subjects ANOVA is relatively robust to violations of this assumption providing the assumption of sphericity is met and the groups are independent, as is the case with these

data. A second 2 x 4 (night x group) ANOVA was used to determine if k-complexes alone changed with new learning. Again, there was no change in k-complexes from baseline to test night as a function of learning condition. Finally, a third 2 x 4 (night x group) ANOVA was used to determine if k-complexes that occur simultaneously with spindles change with new learning. No change in k-complexes that occur simultaneously with spindles was observed from baseline to test night as a function of leaning condition. These results suggest that k-complexes are unaffected by the types of learning used in this experiment. The results from three main k-complex analyses are displayed in table 6.

Rapid Eye Movements

A 2 x 4 (night x group) ANOVA was used to determine if the number of rapid eye movements per minute of REM sleep changed following learning. Contrary to the predictions, there was no interaction of night by learning condition (table 7). There was no violation to the assumptions of normality, homogeneity of variance or sphericity. No outliers were detected in this data set.

Spectral Analysis of Sleep Using FFT

Across all midline sites, 2 x 4 (night x group) ANOVAs were used to test if total sigma power for the entire night changed from baseline to test night as a function of learning condition during Stage 2 sleep. The assumption of homogeneity of variance was violated for total sigma power on test night at recording site Pz only. One statistical outlier was detected on night 2. The outlier was removed. However, even after removing the outlier the assumption remained violated. It was found that night and learning group interacted significantly at Oz only ($F(3, 28) = 4.03$, $p = .017$). A one-way ANOVA on night 1 total sigma for the entire night revealed that the four learning conditions were not

statistically different at baseline ($F(3, 28) = 1.07$, $p = .19$). Interestingly, paired Bonferroni t-tests indicated that the DM group had a significant decrease in total sigma power from baseline to test night for Stage 2 sleep across the entire night ($t(28) = -3.20$, $p < .05$). The S-PM, C-PM, and C groups did not have any change in total sigma power. Contrary to the predictions, there was no increase from baseline to test night in the S-PM group during Stage 2 sleep, however, it is worth noting that sigma power did change in the hypothesized direction, although this effect was not statistically robust.

To determine if the changes in spectral power observed at Oz were limited to the sigma band, the delta, theta, alpha, beta and gamma bands were tested using 2 x 4 (night x group) ANOVAs. There were no significant night by group interactions for any of the other frequency bins. Thus, the change in power during Stage 2 sleep as a result of learning was limited to the sigma band. There was a violation to the assumption of homogeneity of variance for alpha during Stage 2 sleep at Oz. The data were normally distributed, and the assumption of sphericity was met, however, one statistical outlier was detected. After removal of the outlier, the assumption remained violated. The mixed-model ANOVA is robust to violations of this assumption providing the data are normally distributed, and the assumption of sphericity has been met. As described above, Stage 2 sleep total Sigma power at the site of interest (Oz) for each half of the night were analyzed separately. There was no significant learning group by night interaction in the total sigma band for Stage 2 sleep in the first half of the night. However, a 2 x 4 (night x group) ANOVA revealed a significant interaction between night and learning condition in the total sigma band in the second half of the night at Oz ($F(3, 28) = 3.61$, $p = .025$). A simple effects one-way ANOVA revealed that the four learning groups did not differ

significantly in total sigma power on the baseline night ($F(3, 28) = 1.96$, $p = .14$). Again, the DM group had a significant decrease in total sigma power from baseline to test night for Stage 2 sleep in the second half of the night ($t(28) = -2.78$, $p < .05$). The S-PM, C-PM, and C groups had no change in total sigma power. Next, the sigma band was divided into low and high sigma to determine if fast or slow sigma power was of particular importance for memory consolidation during sleep. There was no significant interaction between night and learning condition for high sigma power for Stage 2 sleep in the second half of the night. However, it was found that there was a significant interaction for low sigma power during Stage 2 sleep in the second half of the night ($F(3, 28) = 3.48$, $p = .029$). Follow-up analyses revealed that the groups did not differ significantly in the amount of low sigma power on the baseline night ($F(3, 28) = 2.82$, $p = .057$). Bonferroni t-tests revealed that the DM group had a significant decrease in low sigma power during Stage 2 sleep in the second half of the night from baseline to test night ($t(28) = -3.06$, $p < .05$). There was no change for low sigma power during stage 2 sleep in the second half of the night for the S-PM, C-PM or the C group. Refer to Table 8 for a summary of the results.

Total sigma during SWS was analyzed using a 2 x 4 (night x learning group) ANOVA to determine the effect of learning on total sigma power during SWS at all midline sites. It was found that total sigma during SWS changed as a function of learning condition from baseline to test night at Oz only ($F(3, 28) = 6.28$, $p = .002$). A one-way ANOVA on SWS total sigma power indicated that sigma power of the four groups was not significantly different at baseline ($F(3, 28) = 0.94$, $p = .44$). Follow-up Bonferroni t-tests revealed that total sigma power during SWS increased from baseline to test night in the S-PM group ($t(28) = 2.76$, $p < .05$), and decreased in the DM group ($t(28) = -2.77$, $p <$

.05). Sigma power did not change significantly in the C-PM or C groups. To determine if the changes in spectral power were isolated to the sigma band, the power in the delta, theta, alpha, beta and gamma bands at Oz during SWS were analyzed using 2 x 4 (night x learning group) ANOVAs. There were no significant night by learning group interactions for any frequencies outside of the sigma band at Oz. The assumption of homogeneity of variance was violated for test night delta at Oz during SWS. However, the assumptions of normality and sphericity were met and no outliers were detected. Given this, the robustness of the analysis should not be affected by a violation to the assumption of homogeneity of variance. Using a 2 x 4 (night x learning group) ANOVA to analyze low sigma power during SWS it was found that there was a significant night by learning condition interaction ($F(3, 28) = 8.16$, $p = .00046$). A one-way ANOVA revealed that the four groups did not differ on the baseline night in low sigma power ($F(3, 28) = 1.33$, $p = .28$). Pairwise Bonferroni t-tests revealed that low sigma power during SWS increased in the S-PM ($t(28) = 3.37$, $p < .01$), and decreased in the control group ($t(28) = -2.27$, $p < .05$). Low sigma power during SWS did not change from baseline to the test night in the DM or C-PM groups. Using a 2 x 4 (night x Learning group) ANOVA to analyze high sigma power during SWS it was found that there was a significant night by learning condition interaction ($F(3, 28) = 3.62$, $p = .025$). A one-way ANOVA revealed that the four groups did not differ on the baseline night in high Sigma power ($F(3, 28) = 0.93$, $p = .44$). Pairwise Bonferroni t-tests revealed that high Sigma power during SWS did not change from baseline to the test night in the S-PM, C-PM, DM or C groups. Refer to Table 8 for a summary of the results.

A 2 x 4 (night x learning group) ANOVA was used at each midline site to

determine if theta changed during REM sleep as a function of learning condition from baseline to test night. It was found that there was a significant night by learning group interaction at Cz ($F(3, 28) = 3.27$, $p = .036$), but not at any other midline sites. A one-way simple effects ANOVA for the baseline night only revealed that the four learning groups were not different from one another in theta power at Cz ($F(3, 28) = .98$, $p = .42$). As predicted, paired Bonferroni t-t-test revealed that there was a significant increase in theta power during REM sleep from the baseline to test night for the DM group ($t(28) = 4.52$, $p < .01$). There was no change in theta power for the S-PM, C-PM, or C groups. The assumption of homogeneity of variance was violated for baseline and test night theta at Oz during REM sleep. However, the assumptions of normality and sphericity were met and no outliers were detected. Given this, the analysis should be robust against a violation to the assumption of homogeneity of variance. Additional 2 x 4 (night x learning group) ANOVAs were performed at Cz for the delta, alpha , sigma, beta and gamma bands to determine if the changes in power during REM sleep were isolated to the theta band. Interestingly, there was a significant night by learning condition interaction in the sigma band at Cz ($F(3, 28) = 3.74$, $p = .022$), but not in any other frequency bands. A follow-up simple effects ANOVA for sigma at Cz during REM sleep revealed that the groups were not statistically different at baseline ($F(3, 28) = 0.77$, $p = .52$), and paired Bonferroni t-tests revealed that the PA group had an increase in the sigma band during REM sleep at Cz from baseline to test night ($t(28) = 3.83$, $p < .01$). There was no change from baseline to test night in the S-PM, C-PM and C groups for sigma power during REM sleep at Cz. To determine if low or high sigma was of particular importance to the changes during REM sleep following learning, 2 x 4 (night x learning group) ANOVAs were run using

low sigma power and high sigma power as the dependent variables. It was found that only low sigma power changed from baseline to test night as a function of learning condition ($F(3, 28) = 4.19$, $p = .014$), with no differences at baseline between groups as tested by a one-way simple effects ANOVA ($F(3, 28) = 0.92$, $p = .45$). Follow-up paired Bonferroni t-test revealed that only the DM group had a significant increase in low sigma power during REM sleep at Cz ($t(28) = 4.17$, $p < .01$). The S-PM, C-PM or C groups had no change in low sigma power during REM sleep at Cz. Refer to Table 8 for a summary of the results. Of note, there was a similar pattern of results for high sigma power during REM sleep at Cz that did approach significance ($F(3, 28) = 2.67$, $p = .07$).

Topography of the Learning-dependent Changes in Spectral Power

While the decrease in the sigma band during Stage 2 sleep for the DM group was not as hypothesized, in light of the finding that both theta and sigma power was affected by declarative learning during REM sleep, the topography of this effect was investigated further to provide a more complete picture of the effect of declarative learning on sleep. To summarize from the previous section, it was found that the DM group had a decrease in low sigma power at Oz during the second half of the night Stage 2 sleep, but not in high sigma power, or sigma power during the first half of the night. There was no change in the sigma band during Stage 2 sleep for the S-PM, C-PM, or C groups. Paired t-tests at all active sites for the DM group (Fp1, Fp2, F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, Oz, and O2) revealed that there was a significant decrease in low sigma power in Stage 2 sleep for the last half of the night at Cz ($t(8) = 2.09$, $p = .07$), Pz ($t(8) = 1.94$, $p = .089$), and Oz ($t(8) = 6.59$, $p = .0002$) where the effect was most robust. Table 9 lists all of the means, mean differences and statistical tests from baseline to test night for the DM group

at all active sites during late Stage 2 sleep.

Figure 9 displays low sigma power across the entire scalp during late Stage 2 sleep for each participant included in the FFT analyses on baseline and test night in the DM group only. An important feature to note from these figures is that the upper end of the scale for 6 of the 9 individuals decreases from baseline to test night. These values denote the power (red indicates maximum power, and blue indicates minimum power) in the low sigma band for each individual. Thus, the maximum power in the majority of the individuals in the DM decreased in sigma power from baseline to test night. It is also worth noting that low sigma power was maximal at Pz on baseline ($M = 0.787$, $SD = 0.206$) and test night ($M = 0.741$, $SD = 0.219$), however, the largest decrease in low sigma power was statistically most robust at Oz ($t(8) = 6.59$, $p = .0002$).

In the previous section, it was found that during SWS, low sigma power increased at Oz in the S-PM group but not in any other groups. For the following tests, the data from O2 was excluded from one participant, due to poor quality recordings (impedances over 100 KOhm). Multiple paired t-test at all active sites revealed that there was a significant increase from baseline to test night in low sigma power in the S-PM group during SWS sleep at Fp1 ($t(7) = -2.82$, $p = .026$), Fp2 ($t(7) = -2.49$, $p = .042$), F4 ($t(7) = -2.58$, $p = .036$), Cz ($t(7) = -3.41$, $p = .011$), Pz ($t(7) = -1.96$, $p = .091$), and Oz ($t(7) = -3.11$, $p = .017$). Table 10 lists all of the mean differences and statistical tests from baseline to test night for the S-PM group at all active sites during SWS.

Figure 10 displays the power across the scalp in the low sigma band (12 to 14 Hz) during SWS in the S-PM group on the baseline and test night. Here the distribution of low sigma power is maximal at Cz on baseline ($M = 0.566$, $SD = 0.289$) and test night (M

= 0.588, SD = 0.299). However, the largest increase in low sigma power is at Oz (t(7) = -3.11, p = .017), which indicates a posterior shift in sigma power. In the S-PM group the increase in low sigma power can be observed by the increase in the maximum value of the scale for 5 of the 8 participants in this group from baseline to test night.

From the analyses reported in the previous section it was found that there was an increase in the theta at Cz during REM sleep for the DM group only. Multiple paired t-tests revealed that theta increased from baseline to test night during REM sleep at Fz (t(8) = -2.29, p = .051), F4 (t(8) = -4.97, p = .001), C3 (t(8) = -2.33, p = .048), Cz (t(8) = -5.32, p = .001), C4 (t(8) = -4.28, p = .003), Pz (t(8) = -3.56, p = .007), and O1 (t(8) = -4.22, p = .003). Multiple paired t-tests revealed that sigma increased from baseline to test night during REM sleep at F3 (t(8) = -2.21, p = .058), Fz (t(8) = -2.26, p = .033), F4 (t(8) = -2.51, p = .037), C3 (t(8) = -2.93, p = .019), Cz (t(8) = -3.42, p = .009), C4 (t(8) = -2.05, p = .074) and O1 (t(8) = -2.07, p = .072). Table 11 lists all of the mean differences and statistical tests from baseline to test night for the DM group at all active sites during REM sleep.

Figure 11 displays the distribution of theta power across the scalp for the DM group during REM sleep. Theta is maximal at Cz on baseline (M = 1.010, SD = 0.131) and test night (M = 1.064, SD = 0.116) and the increase from baseline to test night in the DM group is most robust at Cz (t(8) = -5.315, p = .001). The increase in power can be observed in this figure from the maximum values for each scale that denote the highest power in the theta band. In 8 of the 9 individuals, these values increase from baseline to test night. Thus, for the majority of the individuals in the DM group, theta increases from baseline to test following learning.

Power in the low Sigma band during REM sleep for each individual at baseline and test night are shown in Figure 12. The distribution of low sigma power during REM sleep is remarkably different from the distribution of low sigma during Stage 2 sleep or SWS sleep. The power is posterior and more widely distributed across the scalp. REM sleep low sigma power is somewhat less stable within individuals than Stage 2 or SWS, that can be observed in 2 of the 9 individuals (Maps 23, and 41, Figure 12). Low sigma is maximal at O1 on baseline ($M = 0.056$, $SD = 0.197$) and test night ($M = 0.111$, $SD = 0.202$); however, the increase in power is most robust at Cz ($t(8) = -3.421$, $p = .009$), that can be noted as an increase in the maximum power values of 8 of the 9 individuals. Thus, for the majority of the individuals in the DM group, low sigma power increased from baseline to test night following learning.

Discussion

The question posed at the start of this thesis, "What are the functions of sleep?" is a seemingly simple question, however, it is a very difficult to answer. This study cannot provide a direct answer to the question. It can however, provide pieces to the puzzle. The results from this study support the hypothesis that one of the functions of sleep is to consolidate new learning. We have indicated specific stages of sleep that are dissociably related to specific memory types. We have also implicated particular electrophysiological markers that may be mechanisms for sleep-dependent consolidation of memory, and their actions during sleep.

In designing this research, it was decided that simple procedural, cognitive procedural and declarative memory would be used to investigate the effects of learning on sleep. These memory types were chosen based on previous research that has shown

that performance is either sensitive to sleep deprivation (Plihal & Born, 1997; Smith & MacNeill, 1994; Tweed, et al., 1999) or that new learning has an effect on either sleep architecture or phasic activity such as sleep spindles (Fogel, Jacob & Smith, 2002; Gais et al., 2002) or rapid eye movements (Smith et al., submitted). Previous research has shown that simple procedural memory has been found to be impaired following Stage 2 sleep disruption (Smith & MacNeill, 1994). In addition, learning on simple procedural tasks has been found to increase the duration of Stage 2 sleep and increase the density of sleep spindles during Stage 2 sleep (Fogel, Jacob & Smith, 2002). Cognitive procedural memory has been found to be impaired following REM sleep deprivation (Tweed, et al., 1999). Recently, it has been found that learning on complex procedural tasks increases the density of rapid eye movements during REM sleep (Smith et al., submitted). The relationship between declarative memory and sleep is not as clear. Studies have shown that retention intervals predominantly filled with REM sleep are related to improved memory performance (Plihal & Born, 1997). Performance on a declarative memory task was correlated with sigma power during Stage 2 sleep (Gais et al., 2002). Much of this work has been conducted only recently and requires replication. One of the aims of this study was not only to replicate these findings but also to integrate and extend them. The major aims of this study were: (1) to extend previous findings that simple procedural learning increased sleep spindle density in Stage 2, by investigating the effect of learning on slow wave sleep; (2) to determine whether k-complexes were affected by learning; (3) to determine the effect of cognitive procedural and declarative learning on Stage 2 and REM sleep; and (4) to determine the learning dependent topographic changes to sleep EEG that result from new learning.

The Effects of Simple Procedural Learning on Sleep

The first study to investigate the changes in Stage 2 sleep, sleep spindles and Sigma power following simple motor procedural learning found that the duration of Stage 2 sleep, sleep spindle density, and sigma power during Stage 2 sleep increased following an intense period of simple motor procedural learning (Fogel, Jacob & Smith, 2002). There were a number of drawbacks to this study. First, the learning trial consisted of a composite of motor tasks that involved both fine and gross motor control. One of the tasks in this battery of tests was the pursuit rotor. The pursuit rotor was chosen for this study based on the original study (Fogel, Jacob & Smith, 2001), as well as findings that have shown that performance on the pursuit rotor were impaired following Stage 2 sleep disruption (Smith & MacNell, 1994). The difficulty with using a battery of tasks, is that it was not possible to determine which task accounted for the effect or if the tasks interacted with one another in some way. In the current study, only the pursuit rotor was chosen for that reason. Another limitation to the study by Fogel, Jacob and Smith (2001) was that it was not possible to investigate changes to sleep spindle density during SWS. This was due to the inability to display sleep spindles during SWS that were obscured by dominant delta waves. The EEG could only be displayed with the same filters that were used during recording, and no EEG channel displaying only 12 to 16 Hz activity was recorded in this study. In addition, FFT analyses were limited to Stage 2 sleep only. Finally, the last limitation was that the topography of the changes in sigma power during sleep could not be investigated due to an insufficient number of recording sites. The current study has addressed all of these limitations.

Simple procedural learning on the pursuit rotor affected Stage 2 sleep in a number of ways. It was found that the duration of Stage 2 sleep increased from baseline to test night following simple procedural learning, but not cognitive procedural or declarative learning. It was also found that the number of sleep spindles per minute and the duration of sleep spindles increased following simple procedural learning. Sigma power during Stage 2 sleep increased from baseline to test night, however, this result did not reach significance. These findings taken together indicate that Stage 2 sleep is involved in the consolidation of simple procedural learning. Furthermore, the findings suggest that the brain may require additional Stage 2 sleep, with a higher density of spindles that are longer in duration, in order to efficiently encode this new learning into a more permanent form. Thus, thalamocortical input to the cortex increases following learning to consolidate memories during sleep. These findings provide strong evidence that sleep spindles may be the mechanism for the stabilization of these memory traces in the neocortex. The sleep spindle is an ideal mechanism for procedural learning since this type of material requires spaced and repeated stimulation for LTP to occur (Trepel & Racine, 1998; Chapman et al., 1998). The sleep spindle has been suggested to be a mechanism for synaptic plasticity (Steriade & Amzica, 1998; Steriade, 1999).

Contrary to hypotheses, there was no significant increase in sigma power during Stage 2 sleep. This finding was not consistent with the increase in spindle density found during Stage 2 sleep. Several factors could explain this result. Previous findings have shown that late Stage 2 is more closely related to simple procedural memory consolidation than early Stage 2 sleep (Walker et al., 2002). Despite submitting early and late Stage 2 sleep for FFT analysis separately, this still resulted in proportionately more

Stage 2 sleep data submitted for FFT analysis than any other stage of sleep (Table 2). Submitting large amounts of data for FFT analysis may have been problematic for detecting changes in sleep spindles since sleep spindles are not the only source of 12 to 14 Hz activity in Stage 2 sleep. By comparison, tonic 12 to 14 Hz activity would contribute more to the variability in the Sigma band than relatively subtle changes in sleep spindles that are phasic 12 to 14 Hz activity. By submitting smaller amounts of Stage 2 sleep for FFT analysis, the increase in sigma power may have been detected.

Other factors could explain the null Stage 2 sigma power results. EEG oscillates at various frequencies including 12 to 16 Hz. This activity may not be from sleep spindles, or necessarily fluctuate with new simple procedural learning. This non-spindle related 12 to 16 Hz activity would account for a large proportion of power in the sigma band since it is ongoing throughout Stage 2 sleep, and not phasic, like the sleep spindle. Thus, FFT may not be as sensitive as visually detecting spindles, when visual detection is enhanced with the aid of filters. In addition to this, sigma and delta power are inversely related to one another (Aeschbach & Borbely, 1993; Dijk, Hayes & Czeisler, 1993; Uchida, Maloney, March, et al., 1991). One of the characteristic features of SWS is the predominance of delta waves (Rechtschaffen & Kales, 1968). Thus, you would expect sigma power during SWS to be lower than Stage 2 sleep. Therefore, the increase in sigma power following simple procedural learning may be more easily detected during SWS than Stage 2 sleep due to less ongoing tonic 12 to 14 Hz activity that is not spindle related. During Stage 2 sleep Sigma power is higher than in SWS sleep, due to both higher sleep spindle activity and tonic 12 to 14 Hz activity not related to sleep spindles. The increase in sleep spindles during SWS would accordingly cause power in the sigma

band to increase more than during Stage 2 sleep. In a previous study (Fogel, Jacob & Smith, 2002), it was found that sigma power in the 12 to 14 Hz range increased over the course of the entire night during Stage 2 sleep, however, this finding was less robust than the change in sleep spindles. Lastly, the reduction in statistical power resulting from the loss of a participant for the FFT analyses due to poorly recorded EEG may have also contributed to the inconsistency between spindle counting measures and sigma power.

With the advent of new recording techniques, and software that facilitate more flexible offline analyses of EEG, the sleep spindle was measured during SWS by counting sleep spindles with the aid of a filtered EEG channel that displayed activity in the 12 to 16 Hz range. Otherwise, sleep spindles would be obscured by the dominant delta waves that are characteristic of SWS. It was found that sleep spindle density and low sigma power (12 – 14Hz) increased following simple procedural learning, but not cognitive procedural or declarative learning during SWS. This finding taken together with the increase in Stage 2 sleep spindles indicates that sleep spindles are important for the consolidation of new simple procedural learning not only during Stage 2, but also during SWS. Thus, spindles in NREM sleep may be important for the consolidation of simple procedural memory and not just Stage 2 sleep. The finding that slower sigma power (12 – 14Hz), but not faster sigma power (14 – 16Hz), increased following simple procedural learning supported our hypotheses and is consistent with Gilbert's model (2002) which proposed that slower frequencies produce more efficient LTP. This finding was consistent with the hypothesis that slow and fast spindles have separate generators (Merica, 2000), and further, that these frequencies are functionally dissociable. Consistent with predictions, sigma power was maximal at Cz, which is typical for slower

spindle frequencies (Jobert, 1992). While low sigma activity was maximal at Cz, the largest increase in low sigma power from baseline to test night was at Oz. These findings are consistent with the hypothesized increase in low sigma power in centro-parietal and occipital areas that may be active during simple procedural learning such as the motor and visual cortices, respectively. Increased sigma power at occipital regions may be due to increased re-activation of the cerebellum. Gilbert (2002) and others (Grafton et al. 1992; Grafton, Woods, & Tyszka 1994) have implicated the cerebellum in procedural memory consolidation.

The Effects of Cognitive Procedural Learning on Sleep

Cognitive procedural memory has been linked to REM sleep in a number of studies. It has been found that skills that are learned implicitly which require a logical set of rules, such as the Wff'n Proof task (Lapp & Smith, 1986; Smith & Weeden, 1990), are REM dependent. The Wff'n Proof task has been found to be impaired by REM sleep deprivation (Lapp & Smith, 1986). Performance on the Wff'n Proof task has also been found to be enhanced by rapid eye movement coincident stimulation that was also present during training sessions (Smith & Weeden, 1990). Other cognitive procedural memory tasks have been found to be related to REM sleep. Improved performance on the mirror tracing task has been found following intervals filled with REM sleep and Stage 2 sleep in the second half of the night (Plihal & Born, 1997). Performance on the mirror tracing task has been found to be impaired following REM sleep deprivation but not Stage 2 sleep disruption (Tweed et al., 1999). Contrary to the hypothesis that learning on the mirror tracing task would increase REM density, there was no change in REM density following learning in the C-PM, S-PM or DM groups. It is possible that the training

period was not intense enough either in the number of trials or in the difficulty of the task. By narrowing the area between the two lines that the participants must trace within, task difficulty could be increased. Another possibility is that the amplitude criterion for counting eye movements was too strict to detect the effect of learning on rapid eye movements. Most recently, Smith, et al., (submitted) used a more inclusive amplitude criteria of 7 uV compared to the 25 uV criterion used in this experiment.

The Effects of Declarative Learning on Sleep

The study of the relationship between declarative memory and sleep has been fraught with mixed results. Early studies found no effect of total or selective REM sleep deprivation on paired-associates declarative memory performance (Ekstrand et al., 1971; Yaroush et al., 1971; Barrettt & Ekstrand, 1972; Fowler et al. 1972), and have been reviewed by Smith (1995). More recently, it has been suggested that SWS is important for declarative memory performance (Plihal & Born, 1997). They found that performance is better on a paired-associates task when a retention interval was filled primarily with SWS between training and recall. Another study by Gais et al. (2002) has shown that sigma power is correlated with declarative memory performance. Surprisingly, in the current study it was found that declarative learning had an effect on sigma power in both Stage 2 and REM sleep. It was found that low sigma power increased during REM sleep following declarative learning. During Stage 2 sleep, low sigma power decreased following declarative learning. During REM sleep, low sigma power was maximal at occipital sites, whereas the largest increase in power was observed at Cz. During Stage 2 sleep, low sigma power was maximal at parietal sites, and the largest decrease in power was observed at Oz. These findings suggest that low sigma power may oscillate between

Stage 2 sleep and REM sleep following declarative learning whereby low sigma is reduced during Stage 2 sleep and then increases in REM sleep. The former may be driven by a reduction in sleep spindles (Figures 7 & 8), the latter by an increase in sigma power unrelated to the spindle. As Gilbert's model (2001) suggests, depicted in Figure 2, the increase in sigma power during REM sleep may be due to input from the hippocampus to the neocortex that may still stimulate activity in corticothalamic units and stimulate 12 – 14 Hz oscillations, but not at a sufficient level to generate sleep spindles. During REM sleep, there was a shift in power in the low sigma band from occipital sites to central sites following declarative learning. Following simple procedural learning, the shift in Sigma power was from central sites to occipital sites during SWS. Taken together, these results suggest that different brain regions (central vs. occipital) are involved in the consolidation of different types of learning (declarative vs. simple procedural) during different stages of sleep (REM vs. NREM).

Declarative memory is dependent on the hippocampus (Mottoghy et al., 1999) and the basal ganglia (Grafton, Mazziotta, & Presty, 1992; Balslev et al. 2002). Declarative memory requires contextual input and Acetylcholine for LTP to occur. Theta frequency activity is associated with LTP in the hippocampus (Tesche & Karhu, 1978), and predominates during REM sleep (Cantero, Atienza & Stickgold, 2003). Graves, Pack and Abel (2001) suggest that the cholinergic activity in the hippocampus during REM sleep may be related to consolidation of hippocampal-dependent memory. Thus, it was hypothesized that an increase in theta activity would occur during REM sleep. As predicted, there was an increase in theta power following declarative learning, but not after simple procedural or cognitive procedural learning during REM sleep. While it is

known that higher theta during REM is typical compared to other stages of NREM sleep, this study provides the first evidence that indicates that REM sleep theta is involved in the consolidation of declarative memory. This finding suggests that the hippocampus and neocortex may continue to communicate with one another in the theta band to induce LTP for declarative memories. Since the increase in theta is limited to REM sleep this may also indicate that cholinergic activity in the hippocampus during REM sleep may be involved in synaptic plasticity during sleep. It was found that theta was maximal at Cz, and the largest increase in theta was observed at Cz. The topographic stability of the changes in theta power during REM sleep following declarative learning indicate that the areas of the cortex underlying central regions may be involved in the consolidation of new declarative information during REM sleep.

Sleep Spindles and Intelligence

Consistent with previous research (Fogel and Smith, 2003; Nader et al., 2003), and the predictions in this study, a positive relationship was found between baseline sleep spindles and Performance IQ. This finding is consistent with Gilbert's model (2001). Gilbert suggests that the potential to learn is associated with a greater cortical input from thalamocortical units. In the current study, the potential to learn was measured by IQ, and in this case thalamocortical activity was measured by sleep spindles. This relationship was only statistically robust for individuals with high Performance IQ scores (115 and above). Figure 5 illustrates the relationship between Performance IQ and the number of baseline sleep spindles at all three IQ levels (low, medium, and high). It can be seen that there is a linear relationship between Performance IQ and the number of baseline sleep spindles for individuals with a high performance IQ only. Interestingly, it can be seen that

individuals with a very low Performance IQ (below the 24th percentile, equal to an IQ score of 90) have more variability in spindles. It was also found that the increase in Stage 2 sleep spindles following learning was positively related to Performance IQ. This finding suggests that individuals with higher Performance IQ have a larger increase in sleep spindles following new learning. Thus, the effect of new learning on sleep is greater in individuals with a high IQ.

Rapid Eye Movements and Intelligence

Interestingly, it was found that there was a significant positive relationship between Verbal IQ and baseline rapid eye movements for individuals with a high Performance IQ only. This finding suggests that sleep spindles may not be the only electrophysiological marker of the capacity to consolidate new information during sleep. Other phasic activity such as ponto-geniculo-occipital (PGO) waves and rapid eye movements occur in bursting patterns throughout REM sleep (Wu & Siegel, 1990). In cats, the same brain structures that generate PGO activity during REM sleep are associated with the generation of rapid eye movements (Siegel, 2000). PGO activity is generated in the pons (Jouvet, 1962) and propagates to the lateral geniculate nucleus and thalamus (Hobson, Alexander & Frederickson, 1969) to the neocortex. Rapid eye movements have also been found to increase following learning on a two-way shuttle avoidance task in rats (Smith and Lapp, 1986) and following complex procedural learning in humans with high IQ (Smith et al., submitted). This suggests that eye movements during REM sleep are a reflection of higher cognitive processes and are involved in memory consolidation. Figure 6 displays the relationship between Verbal IQ and baseline number of rapid eye movements. This pattern of results is similar to the findings with

sleep spindles and Performance IQ. Again, here it can be seen that individuals with a very low Verbal IQ (below the 16th percentile ie, < 85) are distinct from individuals with a Verbal IQ closer to the normal or high range.

K-Complexes and Memory

Since Stage 2 sleep in general has been implicated in the consolidation of simple procedural and declarative memory it was important to eliminate other phasic activity that characterizes Stage 2 sleep such as k-complexes. There is a controversy about the function of the k-complex. Some believe it is a sleep protective mechanism (inhibitory), while others believe it is an arousal (for review, see: Campbell, Bell & Bastien, 1992). K-complexes have been implicated as being functionally important for sleep maintenance and have been found to be related to information processing during sleep (Oswald, Taylor & Treisman, 1960; Salisbury & Squires, 1993; for review see Bastien, Crowley & Colrain, 2002). In this study there was no change found in k-complex density whether k-complexes occurred concurrently with sleep spindles, without sleep spindles, or both combined. This finding indicates that k-complexes did not change significantly from new learning using the types of memory investigated in this experiment. It may be that k-complexes and sleep spindles are functionally unrelated phasic events in Stage 2 sleep, even when they occur simultaneously. The possibility remains that k-complexes are related to some aspects of memory consolidation during sleep for types of memory that were not tested in this experiment. Alternatively, k-complexes may be related to memory retrieval processes, rather than consolidation of new memory. Evidence for this comes from studies that show the k-complex is evoked more frequently by salient stimuli, for example, one's own name (Oswald, Taylor, & Treisman, 1960).

Limitations of the Current Study

One of the most obvious limitations of the current study was the sample size. While this is a common problem for research of this nature, it can cause a number of problems beyond not having smooth distribution curves and violations of assumptions that underlie the statistical tests used. The lack of power inherent in a small sample size limits the type of dependent variables that can be measured. Only the variables that are responsive to large manipulations in the independent variables that result in large effect sizes can be detected. There may be a number of subtle factors that may go undetected that are of importance, or confound the results.

Many sleep studies attempt to maximize the use of data collected due to the time and labour intensity involved in collecting these data. In addition, any study investigating topographical effects must also consider the effect of conducting numerous statistical analyses on Type I error rates. One way to justify so many tests is to consider the nature of the measures and not simply the number of measures. Most of the dependent variables tested in this experiment are very distinct from one another and normally would not even be investigated in one study (for example the relationship between sleep and IQ, and sleep and memory). One way to conceptualize a study like this to consider it a series of studies using the same subject pool and experimental design. While replication is at the heart of the reliability of experimental results, there is little redundancy in the levels of independent variables (such as learning condition) and in the dependent variables (sleep spindles and rapid eye movements). While there is also considerable overlap in the dependent measures (sleep spindles and sigma power) these measures are used to answer distinct hypotheses, yet also provide strength in the concordance of measures.

Additionally, much care was given in the selection of the statistical analyses and analytic strategies used here that protected against comparison-wise experimental error. For the initial main analyses, the use of the protected test strategy was used to avoid inflating experimental error. Hypothesis-driven analysis of the data was used throughout, and rather conservative multiple comparison techniques such as the Bonferroni t-tests were employed. With the exception of the topographical analysis of the FFT results, Type-I error was kept in check throughout. For these analyses, it was decided that a more liberal statistical approach would be used to gain a more descriptive picture. An important consideration for the use of more liberal criteria was that the topographic analyses were carried out only after the effects were found using more conservative protective tests strategy. Multiple tests are a problem inherent in topographic comparisons. That is why statistical significance is only deemed meaningful when it occurs in logical and circumscribed areas; for example, when predicted by previous research or known underlying neuroanatomy.

Contributions of the Current Study

The current study has strengthened the sleep and memory hypothesis in a number of ways. The finding that Stage 2 sleep spindle density is involved in the consolidation of simple motor procedural memory was replicated here. Importantly, these findings were extended to implicate spindles during SWS also playing a role in sleep-dependent simple procedural memory consolidation. These findings suggest that the sleep spindle has a uniform function across NREM sleep states and not merely Stage 2 sleep. It also implies that the more general categorization of REM and NREM sleep may be a more meaningful distinction for sleep-dependent learning as opposed to the distinction between Stages 2,

SWS and REM. In addition, the results of this study ruled out the possibility of the k-complex as playing a role in the types of memory consolidation investigated here. This suggests that the sleep spindle is a unique phasic event in NREM specific to the consolidation of simple procedural learning.

An important contribution to the literature includes the finding that theta power increased during REM sleep following declarative learning. While it is known that theta is important for communication between the hippocampus and cortex during encoding in wakefulness, there has been no data to this point which has indicated that the same processes occur during sleep. The finding that sigma power during Stage 2 sleep is related to declarative memory (Gais, et al, 2002) was also found in this experiment. Interestingly, it was found that sigma power fluctuated from being significantly lower than baseline in Stage 2 sleep following declarative learning to being significantly higher in REM sleep. In addition, REM sleep was also implicated in declarative memory consolidation in this investigation. Again, this finding highlights the continuity between sleep stages, while still having separate functional roles in memory consolidation.

Another important extension of previous findings is the topographical analysis of the memory-related changes to sleep. In general, it was found that following S-PM learning, low sigma power during SWS was maximal at central sites and had the largest increase at occipital regions. On the other hand, following DM learning, low sigma power during Stage 2 sleep was maximal at parietal sites and decreased at occipital sites. During REM sleep, low sigma power was maximal at occipital sites and increased at central sites following DM learning. In addition, during REM sleep, theta was found to be maximal at central sites where it also had the largest increase. Thus, these findings show that

different types of learning (simple procedural vs. declarative) affect sleep states (NREM vs. REM) in different frequency bands (low sigma vs. theta) in dissociable brain regions (occipital vs. central). These findings suggest that brain plasticity during sleep does not involve a unitary process. In other words, different types of learning have unique sleep-related memory consolidation mechanisms that act in dissociable brain regions at different times throughout the night.

Future Directions

One of the largest limitations in this study is that it was possible that Stage 2 sleep was not split up into small enough sections to detect changes in sigma power during Stage 2 sleep. One approach would be to submit smaller sections of Stage 2 sleep data to FFT analysis. It has been found previously that the second half of the night may be more important for the consolidation of simple procedural learning and that this may be related to sleep spindles (Fogel, Jacob & Smith, 2002; Walker, Brakefield, Seidman, et al., 2002). Submitting Stage 2 sleep by NREM-REM cycle separately may be a more powerful approach to detect differences in sigma power that result from learning-dependent increases in sleep spindles.

In the current investigation, there was no significant increase in rapid eye movements or the duration of REM sleep as a result of cognitive procedural learning. Several factors may have contributed to this. First, training on the task may not have been intense enough to produce a robust change in rapid eye movements. This could be improved on in future research by either increasing the difficulty of the task by changing the parameters of the task (ie; decreasing the distance between the lines to be traced between using the Mirror Tracing task). The intensity of the training could be increased

simply by increasing the number of training trials used before the test night. Other cognitive procedural tasks such as the Tower of Hanoi could be used instead of the tracing task. Second, the criteria for scoring rapid eye movements may have been too conservative in this experiment. Future research may consider using a more inclusive voltage criterion for rapid eye movements.

One of the most longstanding debates in the memory literature has been over how long memory consolidation takes to occur. The current study indicates that this process requires at least a night of sleep, and other research has indicated that even a small amount of sleep no longer than a daytime nap is sufficient to facilitate memory consolidation (Fogel, Milner & Cote, 2004; Mednick, Nakayama & Stickgold, 2003). Future research could easily address this issue simply by measuring parameters such as sleep spindles, rapid eye movements, sigma power and theta power over several nights following an intense period of learning.

In addition, this paradigm could be used to determine if phasic and tonic markers of sleep-related memory consolidation change in aging populations. These parameters may serve as indicators of memory deficits associated with aging. More importantly, age-related changes in sleep quality may be an important factor in age-related changes in memory. Thus, memory deficits and reduced cognitive capacity may be causally related to age-related decreases in Stage 2 sleep, sigma power (Landolt & Borbely, 2001), sleep spindles (Nicolas, Petit, Rompre & Montplaisir, 2001) and possibly other phasic and tonic markers such as rapid eye movements and theta power.

References

Adam, K. & Oswald, I. (1977). Sleep is for tissue restoration. *Journal of Royal College of Physicians of London*, 11, 376 – 388.

Aeschbach, D. & Borbely, A. A. (1993). All-night dynamics of the human sleep EEG. *Journal of Sleep Research*, 70 – 81.

Aeschbach, D., Dijk, D. –J. & Borbely, A. A. (1997). Dynamics of EEG spindle frequency activity during extended sleep in humans: relationship to slow-wave activity and time of day. *Brain Research*, 748, 131 – 136.

Anderer, P., Klosch, G., Trenker, E., Pascual-Marqui, R. D., Zeitlhofer, J., Barbanoj, M. J., et al., (2001). Low-resolution brain electromagnetic tomography revealed simultaneously active frontal and parietal sleep spindle sources in the human cortex. *Neuroscience*, 103(3), 581 - 592.

Anders, T., Sadeh, A. & Appareddy, V. (1995). Normal sleep in neonates and children. In Ferber, R. & Kryger, M. H. (Eds.), Principles and Practice of Sleep Medicine in the Child (pp. 7 – 18). Philidalphia: W.B. Saunders Co.

Asanuma, H., & Pavlides, C. (1997). Neurological basis of motor learning in mammals. *Neuroreport*, 8(4), 1 - 6.

Aserinsky, E., & Kleitman, N. (1953). Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. *Science*, 118, 273 - 274.

Balslev, D., Nielsen, F. A., Frutiger, S. A., Sidtis, J. J., Christiansen, T. B., Svarer, C., et al., (2002). Cluster analysis of activity-time series in motor learning. *Human Brain Mapping*, 15(3), 135-145.

Barrett, T. R., & Ekstrand, B. R. (1972). Effect of sleep on memory: III. Controlling for time-of-day effects. *Journal of Experimental Psychology*, 96(2), 321 -327.

Bastien, C. H., Crowley, K. E., & Colrain, I. M. (2002). Evoked potential components unique to non-REM sleep: relationship to evoked k-complexes and vertex sharp waves. *International Journal of Psychophysiology*, 46, 257 – 274.

Berger, R. J. & Phillips, N. H. (1995). Energy conservation and sleep. *Behavioral Brain Research*, 69(1-2), 65 – 73.

Borbely, A. A. (1982). A two-process model of sleep regulation, *Human Neurobiology*, 1, 195 – 204.

Born, J., & Gais, S. (2003). Roles of early and late nocturnal sleep for the consolidation of human memories. In Maquet, P., Smith, C. & Stickgold, R. (Eds.), *Sleep and Brain Plasticity,* (pp. 65 – 85). Oxford: Oxford University Press.

Buchegger, J., & Meier-Koll, A. (1988). Motor learning and ultradian sleep cycle: an electroencephalographic study of trampoliners. *Perceptual Motor Skills*, 67, 635 - 645.

Buchegger J, Fritsch R, Meier-Koll A, & Riehle H. (1991). Does trampolining and anaerobic physical fitness affect sleep? *Perceptual Motor Skills*, 73(1), 243 – 52.

Campbell, K, Bell, I & Bastien, C. (1992). Evoked Potential Measures of Information Processing During Natural Sleep. In: Broughton, R. & Ogilvie, R. D. (Eds.), *Sleep arousal and Performance,* (pp. 86 - 116). Boston: Birkhauser.

Cantero, J. L., Atienza, M., Stickgold, R., Kahana, M. J., Madsen, J. R. & Kocsis, B. (2003). Sleep-dependent theta oscillations in the human hippocampus and neocortex. *Journal of Neuroscience*. 23(34), 10897-10903.

Carskadon, M. A. & Dement, W. C. (2000). Normal human sleep: an overview. In Kryger, M. H., Roth, T. & Dement, W.C. (Eds.), *Principles and Practice of Sleep Medicine* (pp. 15 – 25). Philadelphia: W.B. Saunders Co.

Chapman, C. A., Trepel, C., Ivanco, T. L., Froc, D. J., Wilson, K., & Racine, R. J. (1998). Changes in the field potentials and membrane currents in rat sensorimotor cortex following repeated tetanization of the corpus callosum in vivo. *Cerebral Cortex,* 8, 730-742.

Cho, Y. H., Beracochea, D., & Jaffard, R. (1993). Extended temporal gradient for the retrograde and anterograde amnesia produced by ibotenate entorhinal cortex lesions in mice. *Journal of Neuroscience*, 13, 1759 - 1766.

Cho, Y. H., & Kesner, R. P. (1996). Involvement of entorhinal cortex or parietal cortex in long-term spatial discrimination memory in rats: retrograde amnesia. *Behavioral Neuroscience*, 110, 436 - 442.

Cleeremans, A. & McClelland, J. L. (1991). Learning the structure of event sequences. *Journal of Experimental Psychology,* 120, 235 – 253.

Cote, K. A., De Lugt, D. R., & Campbell, K. B. (2002). Changes in the scalp topography of event-related potentials and behavioural responses during the sleep onset period. *Psychophysiology*, 39, 29 – 37.

Conway, J., & Smith, C. (1994). REM sleep and learning in humans: a sensitivity to specific types of learning tasks. *12th Congress of the European Sleep Research Society,* Florence, Italy.

Crick, F. & Mitchison, G. (1983). The function of dream sleep. *Nature*. 304(5922), 111 – 4.

Czeisler, C. A. & Khalsa, S. (2000). The human circadian timing system and sleep-wake regulation. In Kryger, M. H., Roth, T. & Dement, W.C. (Eds.), *Principles and Practice of Sleep Medicine* (pp. 353 – 376). Philadelphia: W.B. Saunders Co.

Davis, H., Davis, P. A., Loomis, A. L., Harvey, E. N., Hobart, G. (1939). Analysis of the electrical response of the human brain to auditory stimulation during sleep. *American Journal of Physiology*. 126, 474 – 475.

De Gennaro, L., Ferrara, M., & Bertini, M. (2000). Topographical distribution of spindles: variations between and within NREM sleep cycles. *Sleep Research Online*, 3(4), 155 - 160.

Destexhe, A., & Sejnowski, T. J. (2001). *Thalamocortical assemblies: how ion channels, single neurons and large-scale networks organize sleep oscillations*. New York: Oxford University Press.

Dijk, D. –J., Hayes, B. & Czeisler, C. A. (1993). Dynamics of electroencephalic sleep spindles and slow wave activity in men: Effect of sleep deprivation. *Brain Research*, 626, 190 – 199.

Doran, S. M. (2003). The dynamic topography of individual sleep spindles. *Sleep Research Online*, 5(4): 133 – 139.

Driver, H. (1996). Sleep in Women. *Journal of Psychosomatic Research*, 40(3), 227 - 230.

Ekstrand, B. R., Sullivan, M. J., Parker, D. F., & West, J. N. (1971). Spontaneous recovery and sleep. *Journal of Experimental Psychology*, 88(1), 142 - 144.

Ekstrand, B. R., Barret, T. R., Weat, J. N. & Meier, W. G. (1977). The effect of sleep on human long-term memory. In Drucker-Colin, R. R. & McGaugh, J. L. (Eds.), *Neurobiology of sleep and Memory* (pp. 491 – 438). New York: Academic Press.

Fillmore, M. T. (2003) Reliability of a computerized assessment of psychomotor performance and its sensitivity to alcohol-induced impairment. *Perceptual Motor Skills*. 97(1), 21 – 34.

Fogel, S. (2001). *The effects of simple motor procedural learning on sleep spindle activity in humans*. Unpublished honours thesis, Trent University, Peterborough, Ontario, Canada.

Fogel, S., Jacob, J., & Smith, C. T. (2002). The role of sleep spindles in simple motor procedural learning. *Sleep*, 25, A279 - 280.

Fogel, S. M., Milner, C. E., and Cote, K. A. (2004). Sleep spindles and sigma power predict motor performance improvements following a short nap. Sleep, 27(suppl.): A410-411.

Fogel, S. & Smith, C. (2003). Perceptual and analytical skills account for inter-individual differences in sleep spindles. *Conference Proceedings of the 17th Congress of the European Sleep Research Society*, Prague, 2004.

Fowler, M. J., Sullivan M. J., & Ekstrand, B. R. (1973). Sleep and memory. *Science*, 179, 302 - 304.

Freud, S. (1950). *The Interpretation of Dreams (3rd edition); Translated by A. A. Brill.* New York: Modern Library.

Gais, S., Molle, M., Helms, K., & Born, J. (2002). Learning-dependent increases in spindle density. *The Journal of Neuroscience*, 22(15), 6830 - 6834.

Ghosh, A. & Greenberg, M. E. (1995). Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science*, 268, 239 – 247.

Gibbs, E. L., & Gibbs, F. A. (1962). Extreme spindles: Correlation of electroencephalographic sleep pattern with mental retardation. *Science*, 138, 1106 - 1107.

Gilbert, P. F. C. (2001). An outline of brain function. *Cognitive Brain Research*, 12, 61 - 74.

Glotzbach, S. F. & Heller, H.C. (2000). Temperature Regulation. In Kryger, M. H., Roth, T. & Dement, W.C. (Eds.), *Principles and Practice of Sleep Medicine* (pp. 289 – 304). Philadelphia: W.B. Saunders Co.

Grafton, S. T., Hazeltine, E. & Ivry, R. (1995). Functional mapping of sequence learning in normal humans. *Journal of Cognitive Neuroscience*, 7, 497 – 510.

Grafton, S. T., Mazziotta, J. C., Presty, S., Friston, K. J., Frackowiak, R. S., Phelps, M. E. (1992). Functional anatomy of human procedural learning determined with regional cerebral blood flow and PET. *Journal of Neuroscience*, 12(7), 2542 - 2548.

Grafton, S. T., Woods, R. P., & Tyszka, M. (1994). Functional imaging of procedural motor learning: relating cerebral blood flow with individual subject performance. *Human Brain Mapping,* 1, 221 – 234.

Graves, L., Pack, A., Abel, T. (2001). Sleep and memory: a molecular perspective. *Trends in Neuroscience*, 24(4), 237 – 43.

Grosvenor, A., & Lack, C. L. (1984). The effect of sleep before or after learning on memory. *Sleep*, 7(2), 155 -167.

Gustaffson, B. & Wigstrom, H. (1988). Physiological mechanisms underlying long-term potentiation. *Trends in Neuroscience.* 11(4), 156 – 62.

Hasselmo, M. E. (1999). Neuromodulation: Acetylcholine and memory consolidation. *Trends in Cognitive Sciences,* 3, 351 – 359.

Hebb, D. O. (1966). *A textbook of psychology*. Philadelphia: Saunders.

Hennevin, E., Hars, B., Maho, C., & Bloch, V. (1995). Processing of learned information in paradoxical sleep: relevance for memory. *Behavioural Brain Research*, 69, 125 - 135.

Himanen, S., Virkkala, J., Huhtala, H., & Hasan, J. (2002). Spindle frequencies in sleep EEG show a U-shape within four first NREM sleep episodes. *Journal of Sleep Research*, 11, 35 - 42.

Hobson, J. A. (1998). *Sleep*. New York: Scientific American Library.

Hobson, J. A., Alexander, J. & Frederickson, C. J. (1969). The effect of lateral geniculate lesions on phasic electrical activity of the cortex during desynchronized sleep in the cat. *Brain Research*, 14, 607 – 621.

Horne, J. & Ostberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness. *International Journal of Chronobiology*, 4, 97 – 110.

Howell, D. C. (2001). *Statistical Methods for Psychology*, Belmont, California: Duxbury Press.

Howell, D. C. (2001). Chapter 12: Multiple comparisons among means. In: *Statistical Methods for Psychology.* Belmont, California: Duxbury Press.

Huber, R., Graf, T., Cote, K. A., Wittmann, L., Gallmann, E., Matter, et al. (2000). Exposure to pulsed high-frequency electromagnetic field during waking affects human sleep EEG. *Neuroreport*, 11(15), 3321 - 3325.

Ishizuka, Y., Pollack, C. P., Shirakawa, S., Kakuma, T., Azumi, K., Usui, A., et al., (1994). Sleep spindle frequency changes across menstrual cycle. *Journal of Sleep Research*, 3, 26 - 29.

Ivanco, T. L. & Racine, R. J. (2000). Long-term potentiation in the reciprocal corticohippocampal and corticocortical pathways in the chronically implanted, freely moving rat. *Hippocampus,* 10, 143 – 152.

Jackson, D. N. (1998). *Multidimensional Aptitude Battery-II.* Port Huron, MI: Sigma Assessment Systems, Inc.

Jobert, M., Poiseau, E., Jahnig, P., Schultz, H., & Kubicki, S. (1992). Topographical analysis of sleep spindle activity. *Neuropsychobiology*, 26, 210 - 217.

Karni, A., Meyer, G., Jezzard, P., Adams, M. M., Turner, R., & Ungerleider, L. G. (1995). Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature*. 377(6545), 155 – 8.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science*, 256, 675 - 677.

Kim, J. J., Clarke, R. E., & Thompson, R. F. (1995). Hippocampectomy impairs the memory of recently, but not remotely, acquired trace eye-blink response. *Behavioural Neuroscience*, 109, 195 - 203.

Klimesch, W. (1996). Memory processing, brain oscillations and EEG synchronization. *International Journal of Psychophysiology,* 24, 61-100.

Krueger, J. M. & Obal, F. (1993). A neuronal group theory of sleep function. *Journal of Sleep Research,* 2, 63 – 69.

Landolt, H., & Borbely, A. A. (2001). Age-dependent changes in sleep EEG topography.

Clinical Neurophysiology, 112, 369 - 377.

Landolt, H., Dijk, D., Akermann, P., & Borbely, A. A. (1996). Effect of age on the sleep EEG: slow-wave activity and spindle frequency activity in young and middle-aged men. *Brain Research*, 738, 205 - 212.

Lapp, L., & Smith, C. (1986). The effects of one night's sleep loss on learning and memory in humans. *Sleep Research*, 15, 73.

Larson, J., Wong, D., & Lynch, G. (1986). Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. *Brain Research*, 368, 347-350.

Li, W., Llopis, J., Whitney, M., Zlokarnik, G. & Tsien, R. Y. (1998). Cell-permanent caged InsP3 ester shows that Ca++ spike frequency can optimize gene expression. *Nature,* 392, 936 – 941.

Lucretius (1947). When we sleep. In Woods, R. L. (Ed.), *The World of Dreams* (pp. 188). New York: Random House.

Maquet, P. (2001). The role of sleep in learning and memory. *Science*, 294, 1048.

Maquet, P., Smith, C. & Stickgold, R. (2003). *Sleep and Brain Plasticity.* Oxford: Oxford University Press.

McClelland, J. L., & Rumelhart, D. E. (1988). Explorations in parallel distributed processing: A handbook of models, programs, and exercises. *Computational models of cognition and perception*. Cambridge, MA: The MIT Press.

McDonald, R. J. & White, N. M. (1993). A triple dissociation of memory systems: hippocampus, amygdale and dorsal striatum. *Behavioral Neuroscience,* 107, 3 – 22.

McGaugh, J. L. (2000). Memory – a century of consolidation. *Science*, 287, 248 – 251.

McGrath, M. J., & Cohen, D. B. (1978). REM sleep facilitation of adaptive waking behaviour: A review of the literature. *Psychological Bulletin*, 85(1), 24 - 57.

McGinty, D. & Szymusiak, R. (1990). Keeping cool, a hypothesis about the mechanisms and functions of slow-wave sleep. *Trends in Neuroscience,* 13, 480 – 487.

Mednick, S., Nakayama, K. & Stickgold, R. (2003). Sleep-dependent learning: a nap is as good as a night. *Nature Neuroscience*, 6(7), 697 – 8.

Merica, H. (2000). Fast and slow frequency spindles in sleep: two generators? *Clinical Neuropsychology*, 111, 1704 - 1708.

Mottaghy, F. M., Shah, N. J., Krause, B.J. , Schmidt, D., Halsband, U., Jancke, L., et al. (1999). Neuronal correlates of encoding and retrieval in episodic memory during a paired-word association learning task: a functional magnetic resonance imaging study. *Experimental Brain Research*, 128(3), 332 - 42.

Nadel, L., & Moscovitch, M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex. *Cognitive Neuroscience*, 217 - 227.

Nader, R. & Smith, C. (2003). A role for Stage 2 sleep in memory processing. In Maquet, P, Smith, C. & Stickgold, R. (Eds.), *Sleep and Brain Plasticity* (Chapter 4). Oxford: Oxford University Press.

Nicolas, A., Petit, D., Rompre, S., & Montplaisir, J. (2001). Sleep spindle characteristics in healthy subjects of different age groups. *Clinical Neurophysiology*, 112, 521 - 527.

Ogilvie, R. D. (2001). The process of falling asleep. *Sleep Medicine Reviews*, 5(3), 247 – 270.

Ogilvie, R. D., Simons, I.A., Kuderian, R. H., MacDonald, T., Rustenburg, J. (1991). Behavioral, event-related potential, and EEG/FFT changes at sleep onset. *Psychophysiology*, 28(1), 54 – 64.

Oswald, I., Taylor, A.M. & Treisman, M. (1960). Discrimination responses to stimulation during human sleep. *Brain*, 83, 440–452.

Peigneux, P., Maquet, P., Meulemans, T., Destrebecqz, A., Laureys, S., Degueldre, C., et al., (2000). Striatum forever, despite sequence learning variability: a random effect analysis of PET data. *Human Brain Mapping*. 10(4), 179 – 194.

Peigneux, P., Laureys, S., Fuchs, S., Destrebecqz, A., Collette, F., Delbeuck, X., et al., (2003). Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. *Neuroimage,* 20, 125 – 134.

Plihal, W., & Born, J. (1997). Effects of early and late nocturnal sleep on declarative and procedural memory. *Journal of Cognitive Neuroscience*, 9(4), 534 -547.

Racine, R. J., Milgram, N. W. & Hafner, S. (1983). Long-term potentiation in the rat limbic forbrain. *Brain Research*, 260, 217 – 231.

Rechtschaffen, A. (1998). Current perspectives on the function of sleep. *Perspectives in Biology and Medicine,* 41, 359 – 390.

Rechtschaffen, A., Hauri, P. & Zeitlin, M. (1966). Auditory awakening thresholds in REM and NREM sleep stages. *Perceptual and Motor Skills*. 22(3), 927 – 942.

Rechtschaffen, A., & Kales, A. (1968). *A Manual of Standardized Terminology, Techniques, and Scoring System for Sleep Stage Scoring of Human Subjects*. Bethesda, MD: U. S. Department of Health, Education and Welfare.

Roffwarg, H. P., Muzio, J. N. & Dement, W. C. (1966). Ontogenic development of the human sleep-dream cycle. *Science,* 152, 604 – 618.

Rosenbaum, S., Winocur, G., & Moscovitch, M. (2001). New views on old memories: re-

evaluating the role of the hippocampal complex. *Behavioural Brain Research*, 127, 183 - 197.

Roth, M., Shaw, J., & Green, J. (1956). The form, voltage distribution and physiological significance of the k-complex. *Electroencephalography & Clinical Neurophysiology*. 8, 385 – 402.

Sailisbury, D., & Squires, N. K. (1993). Response properties of long-latency event-related potentials evoked during NREM sleep. *Journal of Sleep Research*, 2(4), 232 - 240.

Schacter, D. L. & Tulving, E. (1994). *Memory Systems*. Cambridge, MA: MIT Press.

Schubotz, R. I. & Von Cramen, D. Y. (2001). Interval and ordinal properties of sequences are associated with distinct premotor areas. *Cerebral Cortex*, 11, 210 – 222.

Seigel, D. (1990). A multivariate study of pursuit rotor skill development. *Research Quarterly Exercise and Sport*. 61(2), 201 – 5.

Siegel, J. M. (2000). Brainstem mechanisms generating REM sleep. In Kryger, M. H., Roth, T. & Dement, W.C. (Eds.), *Principles and Practice of Sleep Medicine* (pp. 112 – 133). Philadelphia: W.B. Saunders Co.

Silverstein, L. D., & Levy, C. M. (1975). The stability of the sigma sleep spindle. *Electroencephalography and Clinical Neurophysiology*, 40, 666 - 670.

Smith, C. (1985). Sleep states and learning: a review of the animal literature. *Neuroscience and Biobehavioural reviews*, 9, 157 - 168.

Smith, C. (1993). REM sleep and learning: some recent findings. In A. Moffit, M. Kramer, & H. Hoffman (Eds.), *The Functions of Dreaming*. Albany: SUNY.

Smith, C. (1995). Sleep states and memory. *Behavioural Brain Research*, 69, 137 – 145.

Smith, C. (2001). Sleep states and memory processes in humans: procedural versus declarative memory systems. *Sleep Medicine Reviews*, 5(6), 491 – 506.

Smith, C. (2003). The REM sleep window and memory processing. In Maquet, P., Smith, C. & Stickgold, R. (Eds.), *Sleep and Brain Plasticity* (chap. 6). Oxford: Oxford University Press.

Smith, C., Conway, J. & Rose, G. (1998). Brief paradoxical sleep deprivation impairs reference, but not working memory in the radial arm maze task. *Neurobiology of Learning and Memory,* 69, 211 – 217.

Smith, C., & Fazekas, A. (1997). Amount of REM sleep and Stage 2 sleep required for efficient learning. *Sleep Research*, 26, 690.

Smith, C. & Lapp, L. (1986). Prolonged increases in both PS and number of REMS following a shuttle avoidance task. *Physiology and Behaviour,* 36, 1053 – 1057.

Smith, C., Lowe, D., & Smith, M. J. (1977). Increases in paradoxical and slow wave sleep during the acquisition of an appetitive task in rats. *Physiological Psychology*, 5, 364 - 372.

Smith, C., & MacNeill, C. (1994). Impaired memory for a pursuit rotor task following Stage 2 sleep loss in college students. *Journal of Sleep Research*, 3, 206 - 213.

Smith, C. T., Nixon M. R., & Nader, R. S. Post-training increases in REM sleep intensity implicate REM sleep in memory processing and provide a biological marker of learning potential. Manuscript submitted for publication.

Smith, C., & Rose, G. M. (1997a). Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiology and Behaviour,* 59, 93 – 97.

Smith, C., & Rose, G. M. (1997b). Posttraining paradoxical sleep in rats is increased after spatial learning in the Morris water maze. *Behavioural Neuroscience*, 111(6), 1197 - 1204.

Smith, C. & Smith, D. (2003). Ingestion of ethanol just prior to sleep onset impairs memory for procedural but not declarative tasks. *Sleep*, 26(2), 185 – 191.

Smith, C., & Weeden, K. (1990). Post training REMs coincident auditory stimulation enhances memory in humans. *Psychiatric Journal of the University of Ottawa*, 15(2), 85 - 90.

Smith, C., Young, J., & Young, W. (1980). Prolonged increases in paradoxical sleep during and after avoidance task acquisition. *Sleep*, 3, 67 - 81.

Squire, L. R. (1998). Memory systems. *C. R. Academie des sciences / Life sciences*, 321, 153 - 156.

Squire, L. R., & Knowlton, B. J. (2000). The medial temporal lobe, the hippocampus, and the memory systems of the brain. In Gazzaniga (ed.), *The Cognitive Neurosciences*, Cambridge, MA: MIT Press.

Squire, L. R., & Zola-Morgan, S. (1996). Structure and function of declarative and nondeclarative memory systems. *Proceedings of the Natural Academy of Science*, 93, 13515 - 13522.

Steriade, M. (1999). Coherent oscillations and short-term plasticity in corticothalamic networks. *Trends in Neuroscience*, 22(8), 337 - 345.

Steriade, M. & Amzica, F. (1998). Coalescence of sleep rhythms and their chronology in corticothalamic networks. *Sleep Research Online*, 1, 1 – 10.

Steriade, M., Jones, E., & Llinas, R. (1990). *Thalamic oscillations and signaling*. New York: John Wiley.

Steriade, M., Kitsikis, A., & Oakson, G. (1979). Selectively REM-related increased firing rates of cortical association interneurons during sleep: possible implications for

learning. In M. A. B. Brazier, (Ed.), *Brain Mechanisms in Memory and Learning From the Single Neuron to Man*. New York: Raven Press.

Steriade, M., McCormick, D. A. & Sejnowski, T. J. (1993). Thalamocortical oscillations in the sleeping and aroused brain, *Science*, 262, 697 – 685.

Stickgold, R., Hobson, J. A., Fosse, R. & Fosse, M. (2001). Sleep, learning and dreams: off-line memory reprocessing, *Science*, 294, 1052 – 1057.

Tabachnick, B. G. & Fidell, L. S. (2001). Computer-Assisted Research Design and Analysis. (pp. 90 – 92). Needham Heights, MA: Allyn & Bacon.

Tesche, C. D., & Karhu, J. (1978). Theta oscillations index human hippocampal activation during a working memory task. *Proceedings of the National Academy of Science*, 97, 919 – 924.

Trepel, C., & Racine, R. J. (1998). Long-term potentiation in the neocortex of the adult, freely moving rat. *Cerebral Cortex,* 8, 719-729.

Tulving, E., Kapur, S., Craik, F. I., Moscovitch, M. & Houle, S. (1994). Hemispheric encoding/retrieval asymmetry in episodic memory: positron emission tomography findings. *Proceedings of the National Academy of Science,* 91(6), 2016 – 2020.

Tweed, S., Aubrey, J. B., Nader, R., & Smith, C. T. (1999). Deprivation of REM sleep or Stage 2 sleep differentially affects cognitive procedural and motor procedural memory. *Sleep*, 22(1), S241.

Tyson, P. D., Ogilvie, R. D. & Hunt, H. T. (1984). Lucid, prelucid, and nonlucid dreams releated to the amount of EEG alpha activity during REM sleep. *Psychophysiology*, 21(4), 442 – 451.

Uchida, S., Maloney, T., March, J D., Azari, R. & Feinberg, I. (1991). Sigma (12 – 15 Hz) and Delta (0.3 – 3 Hz) EEG oscillate reciprocally during NREM sleep. *Brain Research Bulletin,* 27, 93 – 96.

Vallance, K., McDonald, R. J. & Smith, C. (1999). Effects of paradoxical sleep on the memory for a conditioned cue preference task in rats. *Sleep,* 22, S243.

Van De Castle, R. L. (1994). *Our Dreaming Mind.* New York: Ballentine Books.

Walker, M. P., Brakefield, T., Morgan, A., Hobson, J. A. & Stickgold, R. (2002). Practice with sleep makes perfect: Sleep dependent motor skill learning, *Neuron*, 35, 205 – 211.

Walker, M. P., Brakefield, T., Seidman, J., Morgan, A., Hobson, J. A. & Stickgold, R. (2003). Sleep and the time course of motor skill learning. *Learning and Memory*, 10, 275 – 284.

Werth, E. Achermann, P., Dijk, D., & Borbely, A. A. (1997). Spindle frequency activity in the sleep EEG: individual differences and topographic distribution.

Electroencephalography and Clinical Neurophysiology, 103, 535 - 542.

Wiig, K. A., Cooper, L. N., & Bear, M. F. (1996). Temporally graded retrograde amnesia following separate and combined lesions of the perirhinal cortex and fornix in the rat. *Learning and Memory*, 3, 313 - 325.

Williams, R. L. (1966). The first night effect: an EEG study of sleep. *Psychophysiology*, 2(3), 263 – 266.

Winocur, G. (1985). The hippocampus and thalamus: their roles in short and long term memory and the effects of interference. *Behavioural Brain Research*, 16, 135 - 152.

Winocur, G. (1990). Anterograde and retrograde amnesia in rats with dorsal hippocampal or dorsomedial thalamic lesions. *Behavioural Brain Research*, 38, 145 - 154.

Winocur, G., Moscovitch, M., Fogel, S., Rosenbaum, R. S. & Sekeres, M. Preserved spatial memory following hippocampal lesions: effects of extensive experience in a complex environment. (submitted).

Wu, M. F. & Siegel, J. M. (1990). Facilitation of the acoustic startle reflex by ponto-geniculo-occipital waves: effects of PCPS. *Brain Research*, 532, 7 – 17.

Yaroush, R., Sullivan, M. J., & Ekstand, B. R. (1971). Effect of sleep on memory. II: Differential effect of the first and second half of the night. *Journal of Experimental Psychology*, 88(3), 361 - 366.

Yoshitake, H. (1978). Three characteristic patterns of subjective fatigue symptoms. *Ergonomics*, 21, 231 – 233.

Zeitlhofer, J., Gruber, G., Anderer, P., Asenbaum, S., Schimicek, & Saletu, B. (1997). Topographic distribution of sleep spindles in young health subjects. *Journal of Sleep Research*, 6, 149 - 155.

Zepelin, H. (2000). Mammalian sleep. In Kryger, M. H., Roth, T. & Dement, W.C. (Eds.), *Principles and Practice of Sleep Medicine* (pp. 82 – 92). Philadelphia: W.B. Saunders Co.

Zola-Morgan, S., & Squire, L. R. (1990). The primate hippocampal formation: evidence for a time-limited role in memory storage. *Science*, 250, 288-290.

Zola-Morgan, S., & Squire, L. R. (1993). Neuroanatomy of memory. *Annual Review in Neuroscience*, 16, 547- 563.

Table 1
Study design. Participant's IQ is tested followed by one week of recording wake and sleep times in a sleep journal. Acclimatization to the sleep lab occurs on the first night, followed by baseline EEG recording, learning condition, and test night EEG recording. Learning is retested one-week following test night.

	1200 - 1330 hrs	2100 - 2300 hrs	2300 - 0700 hrs
Day 1	MAB-II administration		
Day 2 – 7	Sleep journal		
Night 8	Sleep journal		Acclimatization sleep
Night 9	Sleep journal		Baseline sleep
Night 10	Sleep journal	Learning task/control	Test sleep
Night 17		Learning task re-test	

Table 2
Means and standard deviations of the number of epochs submitted for power spectral analysis using FFT across sleep stages in the simple motor procedural (S-PM), cognitive motor procedural (C-PM), declarative (D) and control (C) groups.

	Baseline Night		Test Night	
	M	SD	M	SD
		Early Stage 2		
S-PM	197.77	31.10	225.67	68.73
C-PM	195.40	27.43	204.07	36.27
DM	170.87	41.70	159.10	46.83
C	178.47	65.50	170.37	49.60
		Late Stage 2		
S-PM	313.73	24.77	302.03	47.53
C-PM	312.00	33.73	283.97	48.30
DM	291.03	48.53	262.20	33.47
C	286.30	37.57	273.53	21.63
		SWS		
S-PM	135.03	50.07	130.87	27.93
C-PM	153.60	41.17	140.87	44.47
DM	178.87	59.73	172.57	54.83
C	154.60	50.30	161.87	32.23
		REM		
S-PM	251.33	64.27	242.30	39.60
C-PM	191.40	50.33	223.87	59.93
DM	226.60	53.83	258.00	35.83
C	239.13	56.93	225.50	44.27

Table 3
Means (M) and standard deviations (SD) for test and re-test on the pursuit rotor (S-PM), mirror tracing task (C-PM), and paired-associates (DM) task.

	Test		Re-test	
	M	SD	M	SD
S-PM	13.01	3.83	15.27*	2.50
C-PM	116.56	38.71	45.00*	24.37
DM	116.44	35.40	149.67*	13.42

Note: Significant differences from pre to post are indicated by * at $p < .005$.

Table 4
Correlation coefficients for the relationship between IQ scores across IQ ranges with the number of Stage 2 baseline night spindles (Spindles), REM sleep rapid eye movements (REMs), the change in the number of Stage 2 sleep spindles (Spindles) and the change in REM sleep rapid eye movements (REM).

	Low IQ			Medium IQ			High IQ			Full range IQ		
	r	p	n	r	p	n	r	p	n	r	p	n
Verbal IQ												
Spindles	0.18	0.53	14	-0.28	0.43	10	0.12	0.73	11	0.01	0.94	35
REMs	0.50	0.07	14	0.38	0.28	10	0.65	0.03*	11	0.26	0.14	35
Spindles	0.18	0.55	14	0.00	0.99	10	-0.24	0.48	11	0.25	0.16	35
REM	0.15	0.60	14	-0.06	0.87	10	0.09	0.78	11	0.13	0.47	35
Performance IQ												
Spindles	-0.17	0.61	12	0.26	0.42	12	0.75	0.01*	11	0.05	0.79	35
REM	0.42	0.17	12	0.24	0.45	12	0.25	0.45	11	0.09	0.61	35
Spindles	0.43	0.16	12	0.37	0.24	12	-0.27	0.43	11	0.36	0.03*	35
REM	0.55	0.06	12	0.08	0.80	12	0.10	0.78	11	0.08	0.67	35
Full Scale IQ												
Spindles	0.23	0.47	12	0.17	0.56	14	-0.09	0.81	9	0.00	0.98	35
REM	0.42	0.169	12	0.30	0.30	14	0.40	0.29	9	0.17	0.33	35
Spindles	0.21	0.518	12	-0.50	0.07	14	-0.28	0.47	9	0.34	0.04*	35
REM	0.36	0.249	12	0.26	0.36	14	0.34	0.38	9	0.12	0.50	35

Note: * indicates a significant correlation at $p < .05$.

Table 5
Means (M) and standard deviations (SD) from ANOVA on minutes of sleep Stages 1, 2, SWS, and REM for the simple procedural learning (S-PM) group, cognitive procedural learning (C-PM), declarative learning (DM) and (C) control groups on the baseline night compared to the test night.

	Baseline Night		Test Night	
	M	SD	M	SD
		S-PM		
Stage 1	6.3	5.2	5.8	3.7
Stage 2	250.8	18.6	268.5*	25.3
SWS	74.2	18.1	39.8	13.0
REM	111.5	27.2	62.4	15.0
		C-PM		
Stage 1	13.0	14.5	12.6	17.3
Stage 2	251.7	25.7	238.1	33.2
SWS	76.2	17.9	51.1	13.4
REM	95.7	21.7	74.7	22.1
		DM		
Stage 1	12.2	18.3	10.1	5.7
Stage 2	220.9	33.2	203.0	35.1
SWS	89.6	29.0	55.3	24.5
REM	106.3	31.1	87.8	27.9
		Control		
Stage 1	9.4	7.3	20.1	12.4
Stage 2	233.0	21.8	209.6	24.6
SWS	78.6	23.7	50.3	17.3
REM	102.7	22.9	79.3	16.9

Note. Significant differences from control group within a sleep stage are indicated by * $p < .05$.

Table 6
Means and standard deviations of the number of k-complexes per minute in Stage 2 sleep for the simple procedural memory (S-PM), complex procedural memory (C-PM), declarative memory (DM), and control (C) groups on the baseline and test nights. Total k-complexes, k-complexes in the absence of spindles, and k-complexes that occur concurrently with spindles are displayed separately.

	Baseline		Test	
	M	SD	M	SD
		Total k-complexes		
S-PM	2.51	1.01	2.66	1.37
C-PM	2.40	0.73	2.28	0.90
DM	2.58	0.80	2.77	0.83
C	2.73	0.93	2.74	0.70
		k- complexes alone		
S-PM	0.86	0.49	0.90	0.59
C-PM	0.89	0.41	0.84	0.34
DM	1.03	0.31	1.15	0.39
C	1.08	0.47	0.97	0.45
		k- complexes with spindles		
S-PM	1.65	0.64	1.77	1.00
C-PM	1.50	0.47	1.44	0.60
DM	1.55	0.59	1.61	0.66
C	1.65	0.60	1.76	0.63

Table 7
Means and standard deviations of the number of rapid eye movements per minute in REM sleep for the simple procedural memory (S-PM), complex procedural memory (C-PM), declarative memory (DM), and control (C) groups on the baseline and test nights.

	Baseline		Test	
	M	SD	M	SD
S-PM	7.26	3.35	7.75	4.11
C-PM	6.65	2.44	7.34	2.75
DM	6.71	2.18	6.32	3.38
C	8.39	3.61	9.12	2.80

Table 8
Summary of significant night by learning group interactions for late Stage 2 sleep, and SWS at Oz, REM sleep low Sigma, and REM sleep theta power at Cz.

	Baseline Night		Test Night	
	M	SD	M	SD
	Oz Late Stage 2 low Sigma power			
S-PM	0.38	0.09	0.42	0.09
C-PM	0.47	0.17	0.45	0.17
DM	0.48	0.15	0.40*	0.18
C	0.30	0.15	0.27	0.13
	Oz SWS low Sigma power			
S-PM	0.28	0.14	0.32*	0.14
C-PM	0.31	0.14	0.30	0.13
DM	0.29	0.23	0.25	0.24
C	0.16	0.10	0.13*	0.10
	Cz REM sleep Theta power			
S-PM	1.06	0.17	1.07	0.19
C-PM	0.94	0.16	0.95	0.16
DM	1.01	0.13	1.06**	0.12
C	1.05	0.15	1.06	0.14
	Cz REM sleep low Sigma power			
S-PM	-0.12	0.19	-0.11	0.22
C-PM	-0.25	0.20	-0.23	0.18
DM	-0.25	0.23	-0.15*	0.26
C	-0.19	0.10	-0.21	0.14

Note. Significant differences indicated by * at $p < .05$ and ** at $p < .01$ using paired Bonferroni t-tests and pooled MSerror with df = 28 for all tests.

Table 9
Topographical mean differences, standard error (SE), paired t-tests (t) and probability values (p) from baseline to test night across all active sites for Stage 2 sleep low Sigma power in the DM group.

Site	Baseline (M)	Test (M)	Mean difference	SE of the mean difference	t	p
Fp1	-0.24	-0.29	0.06	0.04	1.45	0.19
Fp2	-0.32	-0.32	0.00	0.05	0.01	0.99
F3	0.38	0.34	0.04	0.03	1.37	0.21
Fz	0.36	0.34	0.01	0.03	0.46	0.66
F4	0.33	0.33	0.00	0.01	-0.04	0.97
C3	0.57	0.55	0.02	0.03	0.87	0.41
Cz	0.75	0.73	0.02	0.01	2.09	0.07*
C4	0.46	0.46	0.00	0.02	0.04	0.97
P3	0.51	0.47	0.04	0.02	1.70	0.13
Pz	0.79	0.74	0.05	0.02	1.94	0.09*
P4	0.40	0.36	0.04	0.03	1.40	0.20
O1	0.46	0.44	0.02	0.02	1.21	0.26
Oz	0.48	0.40	0.08	0.01	6.59	0.00**
O2	0.28	0.36	-0.08	0.11	-0.73	0.49

Note: Significant change in power is indicated by ** at $p < .001$ and * at $p < .10$, with 8 df.

Table 10
Topographical mean differences, standard error (SE), paired t-tests (t) and probability values (p) from baseline to test night across all active sites for SWS low Sigma power in the S-PM group.

Site	Baseline (M)	Test (M)	Mean difference	SE of the Mean difference	t	p
Fp1	-0.35	-0.29	-0.07	0.02	-2.82	0.03**
Fp2	-0.40	-0.34	-0.06	0.02	-2.49	0.04**
F3	0.22	0.22	0.00	0.03	0.04	0.97
Fz	0.28	0.29	-0.01	0.02	-0.46	0.66
F4	0.19	0.27	-0.08	0.03	-2.58	0.04**
C3	0.36	0.35	0.00	0.02	0.07	0.95
Cz	0.57	0.59	-0.02	0.01	-3.41	0.01**
C4	0.27	0.32	-0.05	0.03	-1.72	0.13
P3	0.22	0.21	0.01	0.02	0.56	0.59
Pz	0.48	0.50	-0.03	0.01	-1.96	0.09*
P4	0.17	0.18	-0.02	0.02	-0.67	0.53
O1	0.26	0.27	-0.01	0.02	-0.44	0.67
Oz	0.28	0.32	-0.04	0.01	-3.13	0.02**
O2	0.24	0.26	-0.03	0.02	-1.49	0.19

Note: Significant change in power is indicated by ** at $p < .05$ and * at $p < .10$, with 7 df. Site O2 was tested with 6 df.

Table 11
Topographical mean differences, standard error (SE), paired t-tests (t) and probability values (p) from baseline to test night across all active sites for REM sleep Theta and low Sigma power in the DM group.

Site	Baseline (M)	Test (M)	Mean difference	SE of the mean difference	t	p
			Theta			
Fp1	0.03	0.06	-0.04	0.02	-1.71	0.13
Fp2	-0.05	-0.03	-0.02	0.03	-0.80	0.45
F3	0.48	0.51	-0.03	0.02	-1.37	0.21
Fz	0.58	0.64	-0.05	0.02	-2.29	0.05*
F4	0.42	0.48	-0.06	0.01	-4.97	0.00+
C3	0.74	0.79	-0.04	0.02	-2.33	0.05**
Cz	1.01	1.06	-0.05	0.01	-5.32	0.00+
C4	0.70	0.75	-0.05	0.01	-4.28	0.00+
P3	0.81	0.83	-0.03	0.05	-1.59	0.15
Pz	0.94	0.98	-0.04	0.01	-3.56	0.01+
P4	0.81	0.82	-0.01	0.01	-0.48	0.64
O1	0.97	1.01	-0.04	0.01	-4.22	0.00+
Oz	0.99	1.01	-0.02	0.01	-1.51	0.17
O2	0.83	0.98	-0.15	0.12	-1.19	0.27
			Low Sigma			
Fp1	-0.93	-0.90	-0.03	0.03	-1.00	0.35
Fp2	-0.93	-0.87	-0.06	0.04	-1.46	0.19
F3	-0.64	-0.55	-0.09	0.04	-2.21	0.06*
Fz	-0.63	-0.52	-0.11	0.04	-2.56	0.03**
F4	-0.67	-0.58	-0.09	0.03	-2.51	0.04**
C3	-0.43	-0.31	-0.12	0.04	-2.93	0.02**
Cz	-0.26	-0.15	-0.11	0.03	-3.42	0.01+
C4	-0.41	-0.34	-0.07	0.03	-2.05	0.07*
P3	-0.23	-0.18	-0.05	0.03	-1.63	0.14
Pz	-0.08	-0.02	-0.06	0.04	-1.29	0.23
P4	-0.21	-0.18	-0.03	0.02	-1.66	0.14
O1	0.06	0.11	-0.06	0.03	-2.07	0.07*
Oz	0.02	0.04	-0.02	0.01	-1.06	0.32
O2	-0.07	0.06	-0.13	0.10	-1.34	0.22

Note: Significant change in power is indicated by $^{+}$ at $p < .01$, ** at $p < .05$ and * at $p < .10$, with 8 df.

Figure 1. Typical "ideal" or "average" pattern of the non-REM/REM sleep cycle across a full, 8-hour night of sleep in a healthy young adult.

Figure 2. Neural network of structures outlined to be important for declarative memory (hippocampus), cognitive procedural memory (basal ganglia), and simple procedural memory (cerebellar-thalamo-cortical). Corticothalamic units are highlighted in red (afferent) and blue (efferent). Plasticity occurs at cortical layer 2/3 and the connections from the thalamus that makes up a memorizing group of cells.

Figure 3. Thalamocortical loops. Neurons in the thalamus (green) project to cortical (blue) layer IV neurons which synapse with layer II/III neurons. Layer V/VI neurons project back to the thalamus to form thalamocortical loops. Interneurons (red) serve as regulatory gating mechanisms to prevent over-stimulation within the loops. Source: taken from: http://info.med.yale.edu/neurobio/mccormick/seminar/figure3.htm.

Figure 4. Schematic representation of the connections between functional networks of brain structures involved in memory consolidation.

Figure 5. Scatterplot of Performance IQ and baseline night sleep spindles in the low (square), medium (triangle) and high (circle) IQ groups.

Figure 6. Scatterplot of Verbal IQ and baseline night rapid eye movements in the low (square), medium (triangle) and high (circle) IQ groups.

Figure 7. Increased sleep spindle density from baseline to test night after simple motor procedural learning during Stage 2 sleep. Note: * indicates $p < .05$.

Figure 8. Increased sleep spindle duration from baseline to test night after simple motor procedural learning during Stage 2 sleep. Note: * indicates $p < .01$.

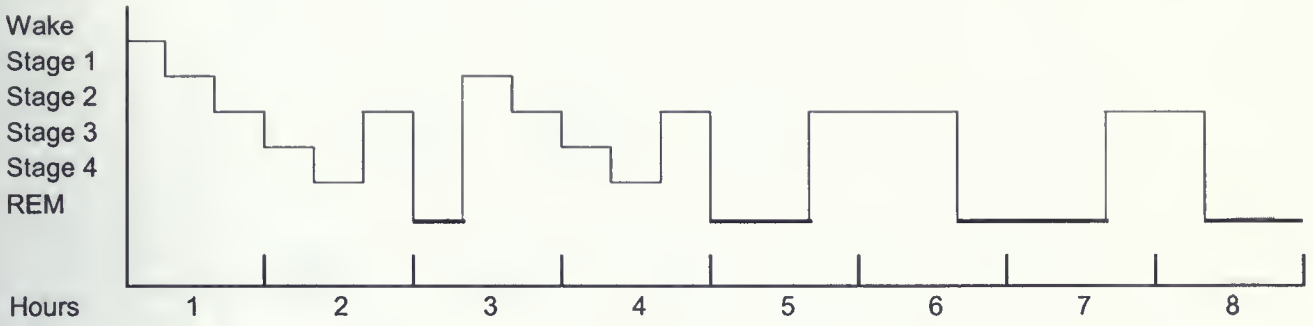
Figure 9. Topographic changes in low Sigma power (12 to 14 Hz) from baseline (top panel) to test night (bottom panel) for each participant individually in the DM group during late Stage 2 sleep. The Spyder software used to generate these maps automatically scales the maps with a fixed gradient from maximum power (red) to minimum power (blue). To interpret changes in power from baseline to test night within an individual, the maximum value for each scale must be considered. Thus, an increase in power is reflected by the increase in the scale for each map.

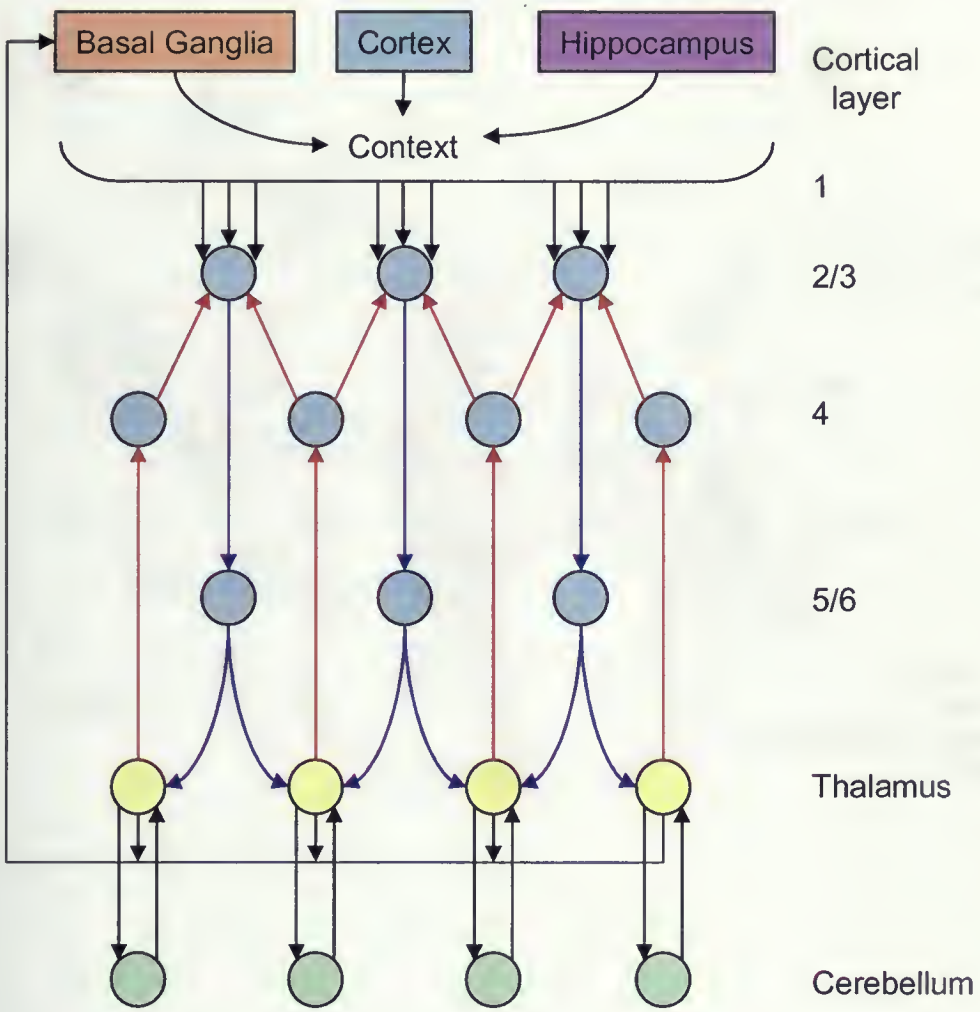
Figure 10. Topographic changes in low Sigma power (12 to 14 Hz) from baseline (top panel) to test night (bottom panel) for each participant individually in the S-PM group during SWS sleep. The Spyder software used to generate these maps automatically scales the maps with a fixed gradient from maximum power (red) to minimum power (blue). To interpret changes in power from baseline to test night within an individual, the maximum value for each scale must be considered. Thus, an increase in power is reflected by the increase in the scale for each map.

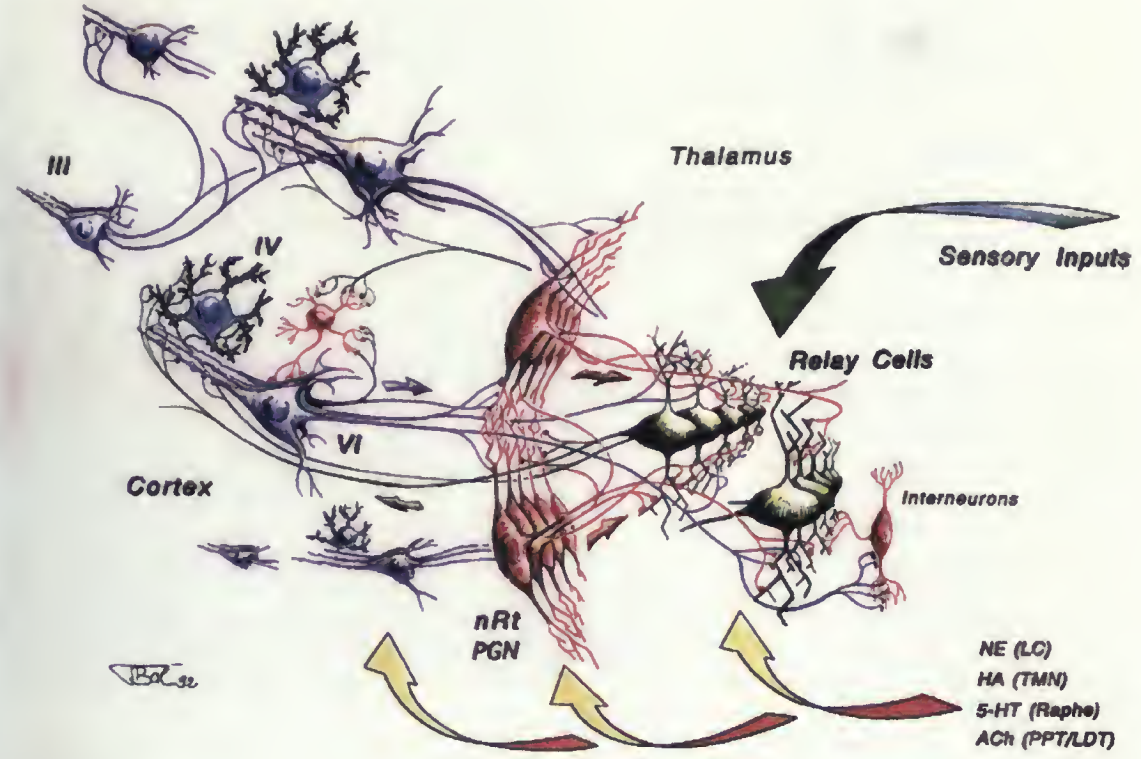
Figure 11. Topographic changes in Theta power (4 to 8 Hz) from baseline (top panel) to test night (bottom panel) for each participant individually in the DM group during REM sleep. The Spyder software used to generate these maps automatically scales the maps with a fixed gradient from maximum power (red) to minimum power (blue). To interpret changes in power from baseline to test night within an individual, the maximum value for each scale must be considered. Thus, an increase in power is reflected by the increase in the scale for each map.

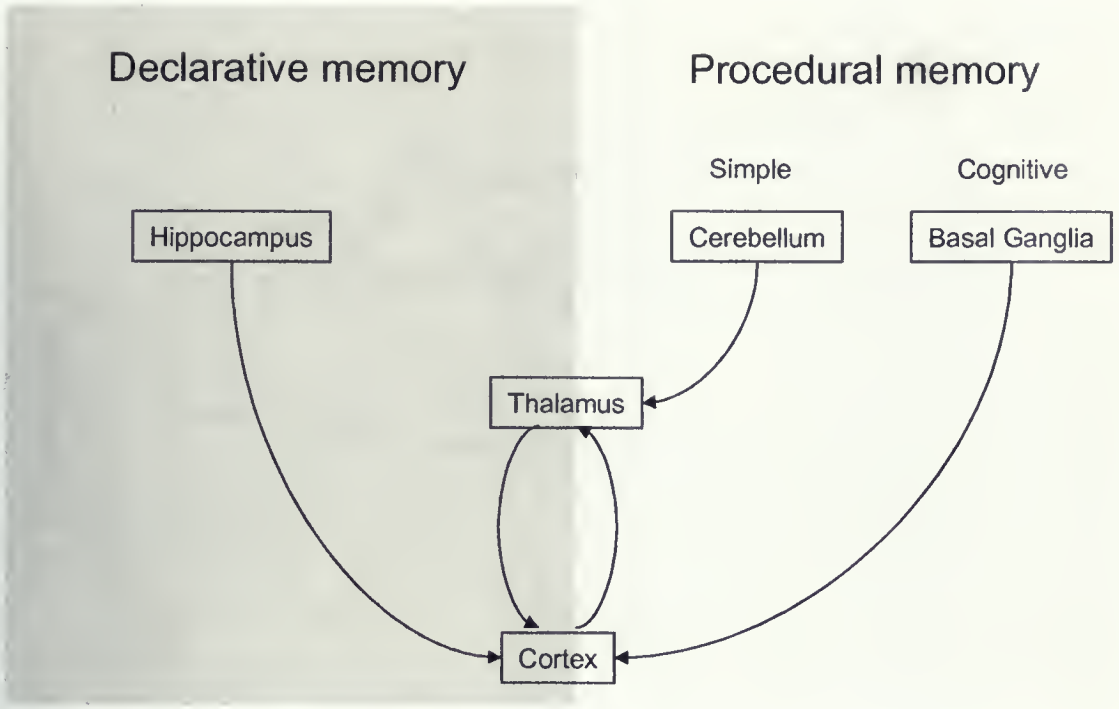
Figure 12. Topographic changes in low Sigma power (12 to 14 Hz) from baseline (top panel) to test night (bottom panel) for each participant individually in the DM group during REM sleep. The Spyder software used to generate these maps automatically scales the maps with a fixed gradient from maximum power (red) to minimum power (blue). To interpret changes in power from baseline to test night within an individual, the maximum value for each scale must be considered. Thus, an increase in power is reflected by the increase in the scale for each map.

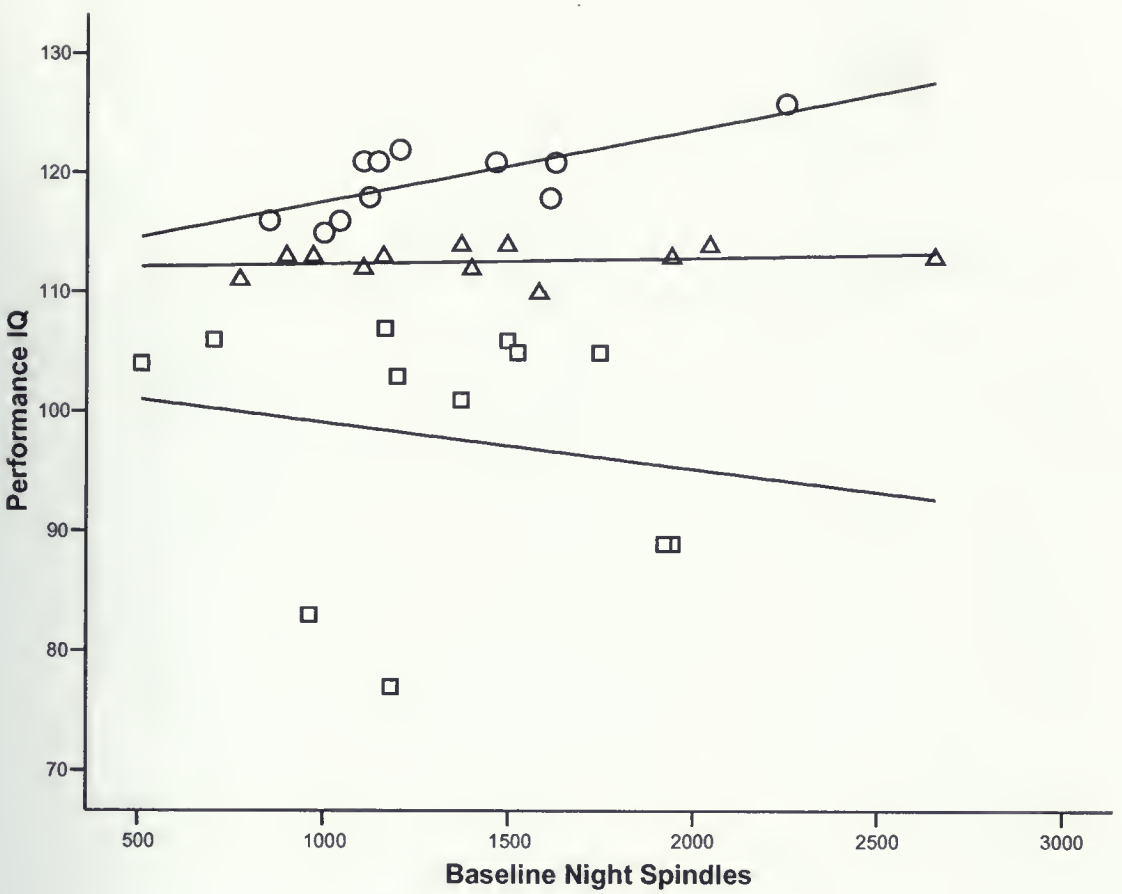
Figures

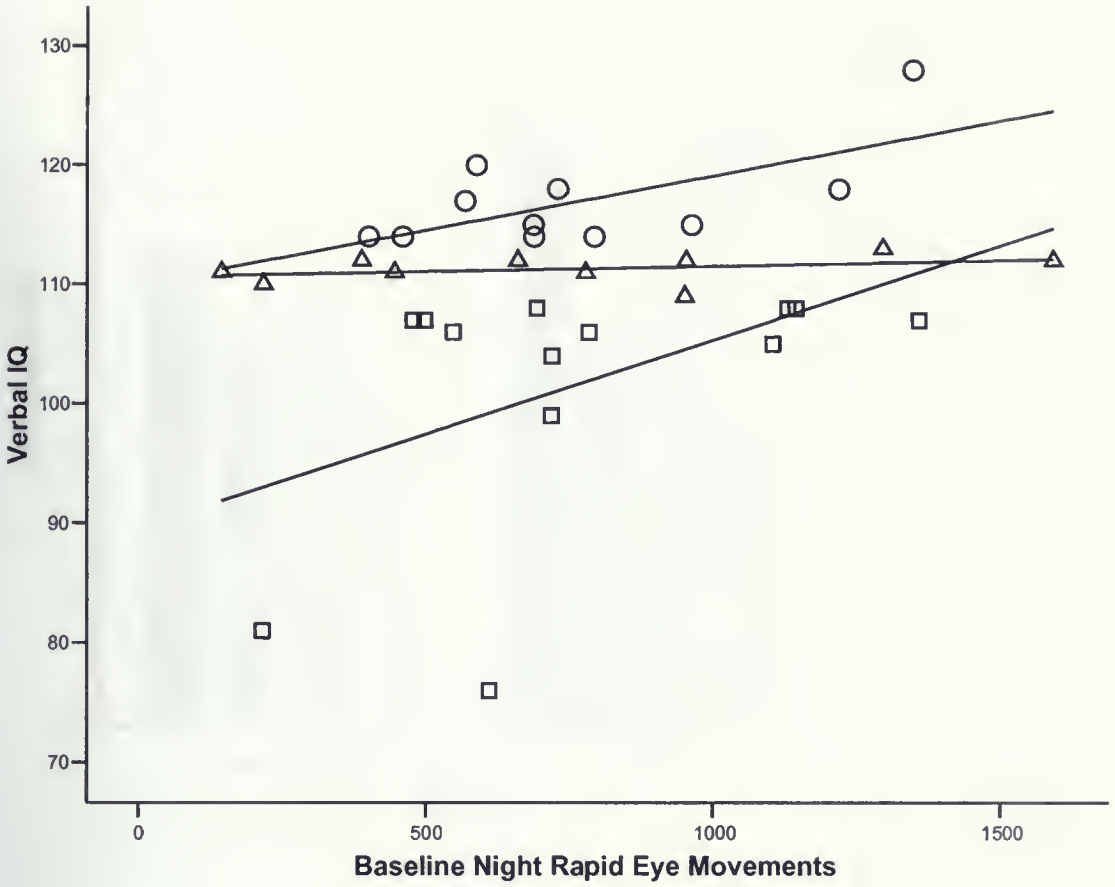


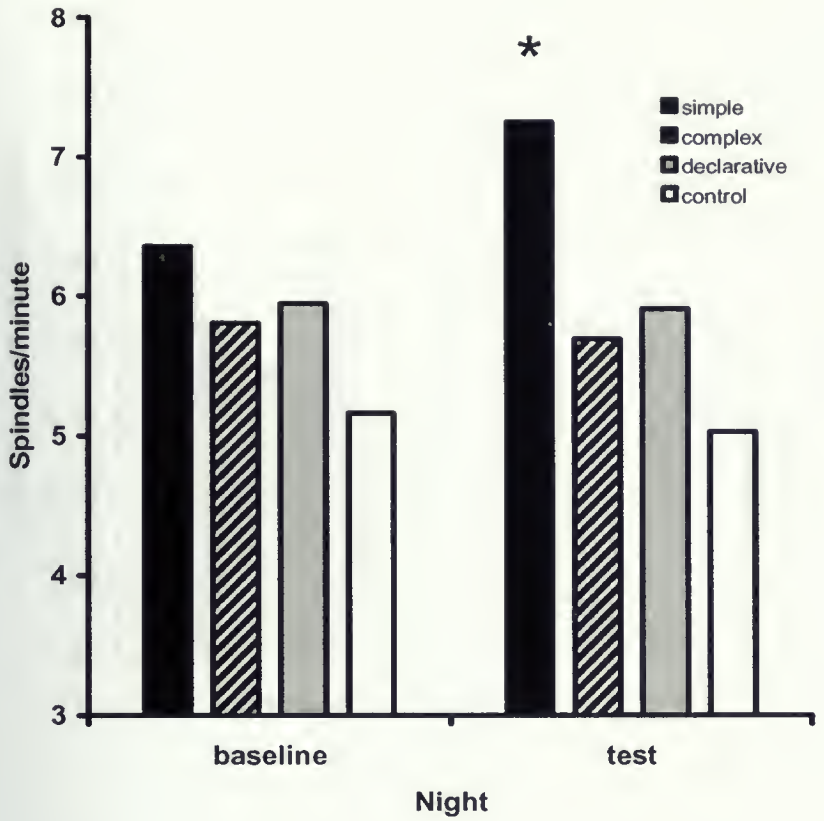

Basal Ganglia
Cortex
Hippocampus
Context
Cortical layer
1
2/3
4
5/6
Thalamus
Cerebellum

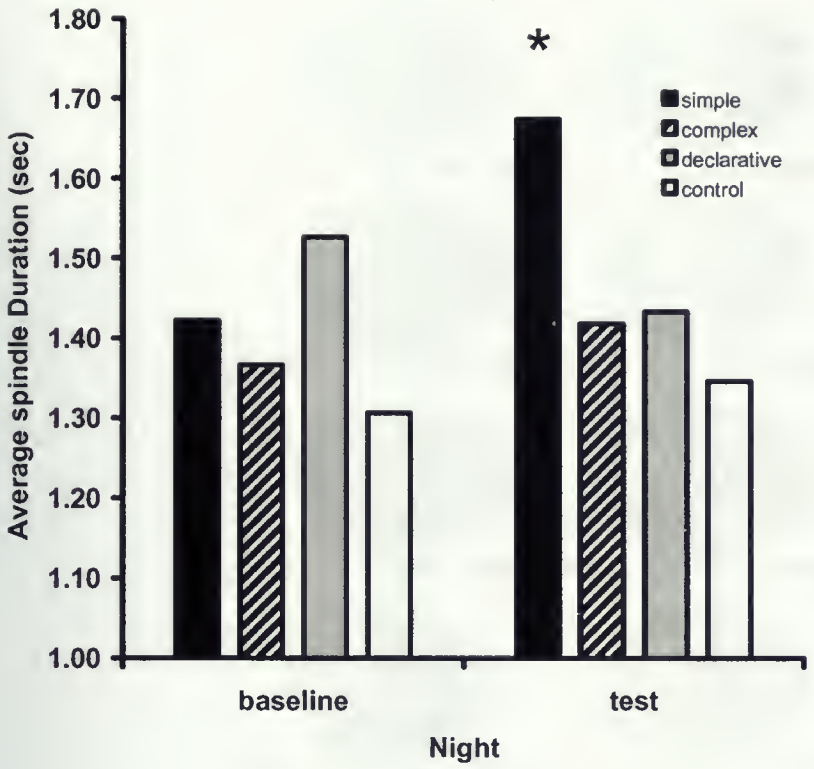

III
IV
VI
Cortex
Thalamus
Sensory Inputs
Relay Cells
Interneurons
nRt
PGN
NE (LC)
HA (TMN)
5-HT (Raphe)
ACh (PPT/LDT)

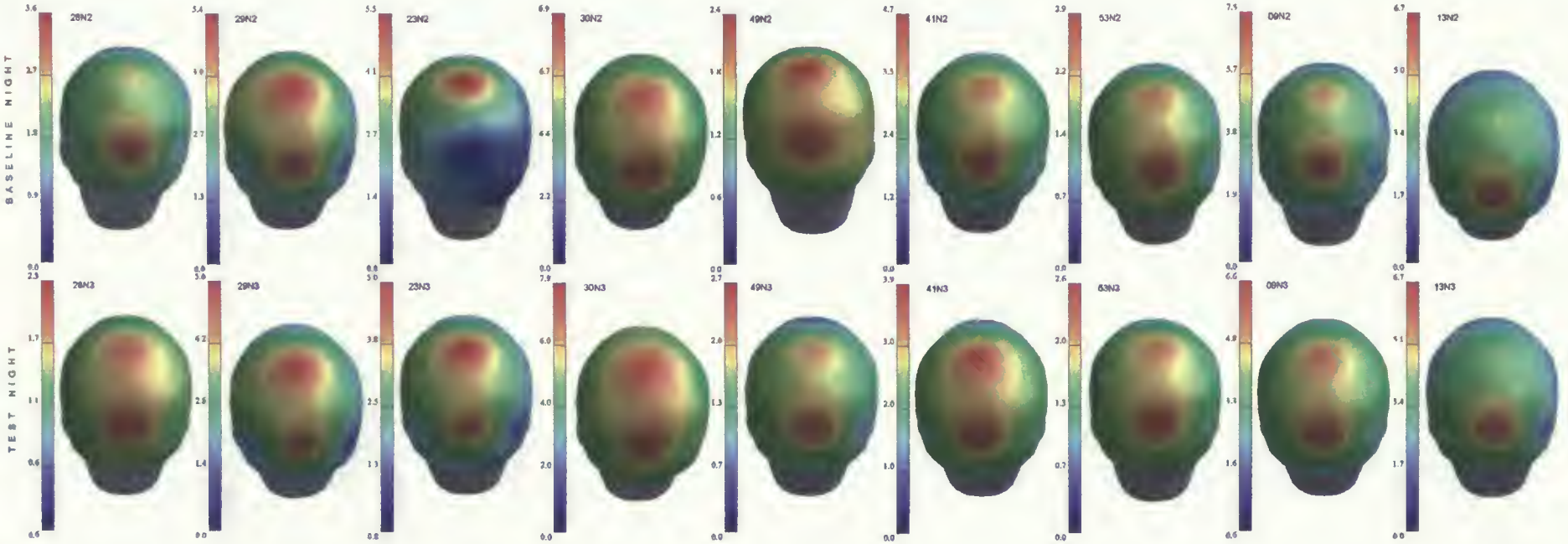

Declarative memory
Procedural memory
Simple
Cognitive
Hippocampus
Cerebellum
Basal Ganglia
Thalamus
Cortex


Performance IQ
130
120
110
100
90
80
70
500
1000
1500
2000
2500
3000
Baseline Night Spindles


130
120
110
100
90
80
70
Verbal IQ
0
500
1000
1500
Baseline Night Rapid Eye Movements


8
7
6
5
4
3
Spindles/minute
*
simple
complex
declarative
control
baseline
test
Night


1.80
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
Average spindle Duration (sec)
*
simple
complex
declarative
control
baseline
test
Night


BASELINE NIGHT
TEST NIGHT
28N2
29N2
23N2
30N2
49N2
41N2
53N2
09N2
13N2
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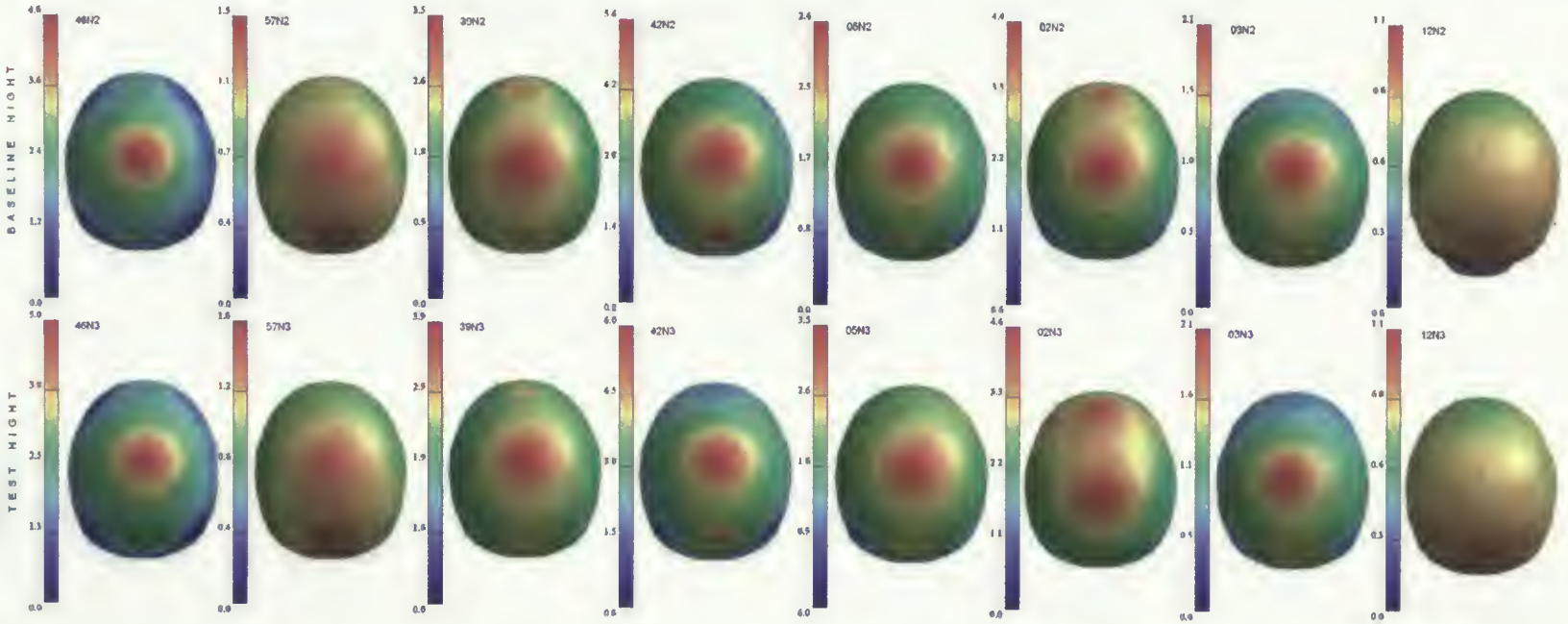
30N3
49N3
41N3
53N3
09N3
13N3


BASELINE HIGHT
TEST HIGHT
46N2
57N2
39N2
42N2
08N2
02N2
03N2
12N2
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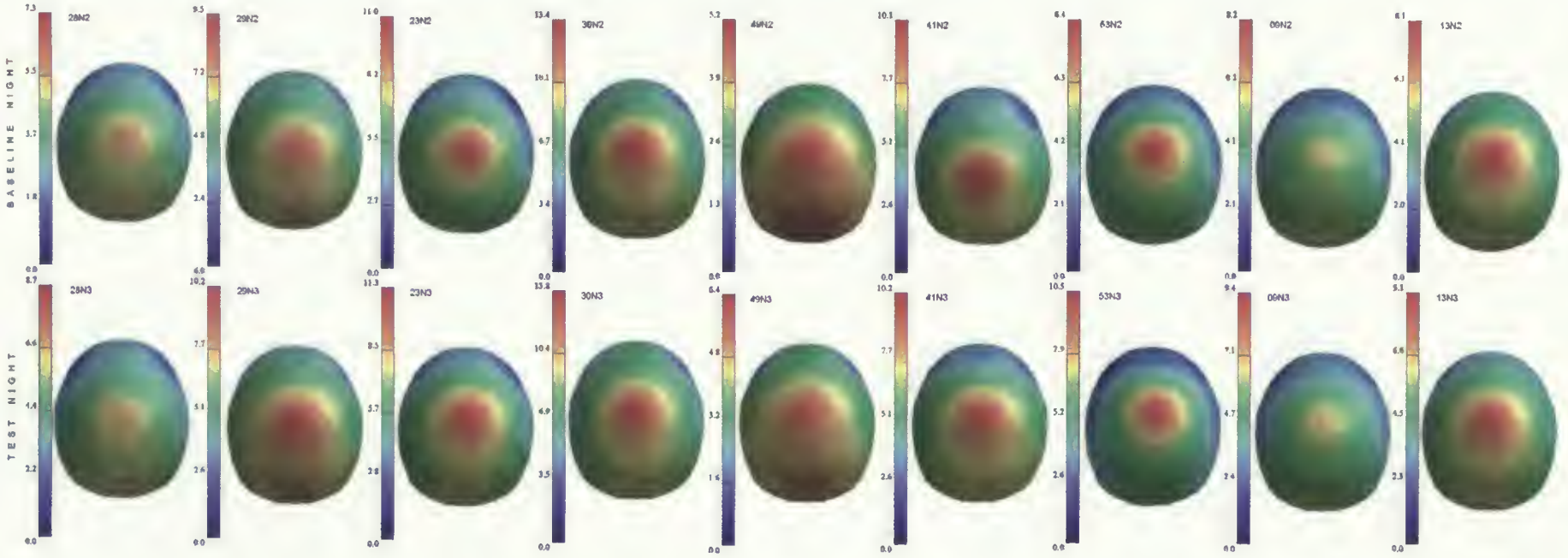
39N3
42N3
05N3
02N3
03N3
12N3


BASELINE NIGHT
TEST NIGHT
28N2
29N2
23N2
30N2
46N2
41N2
63N2
09N2
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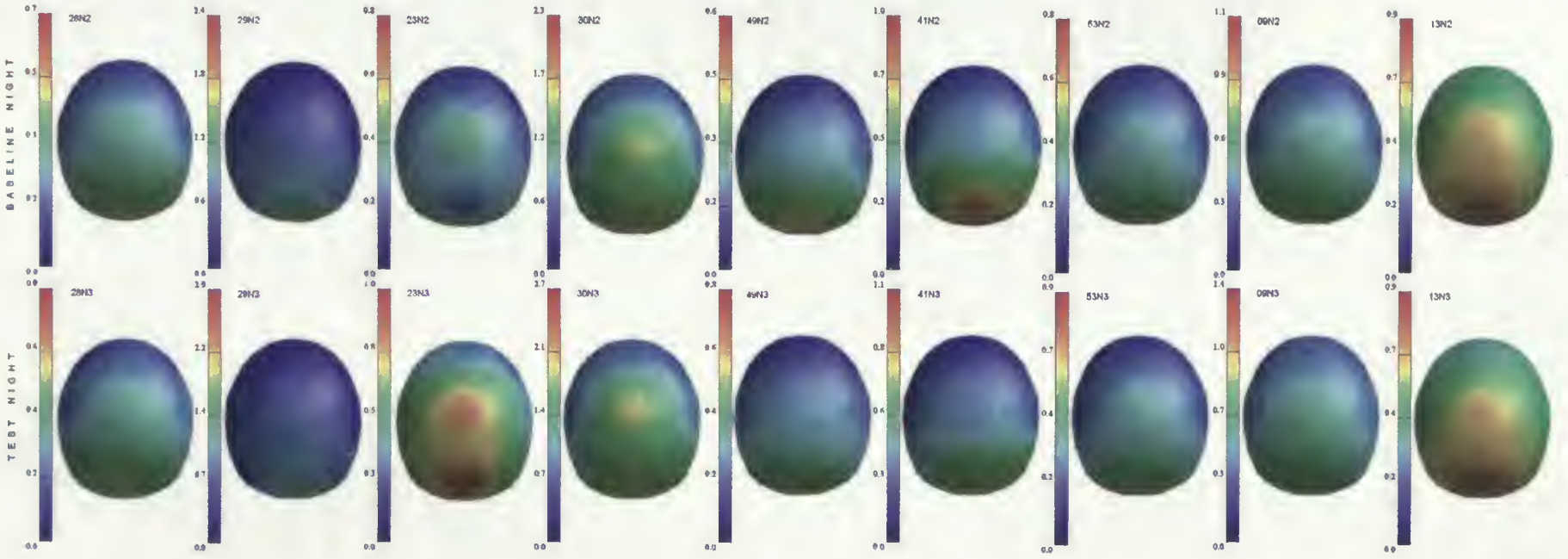
30N3
49N3
41N3
53N3
09N3
13N3


BASELINE NIGHT
TEST NIGHT
28N2
29N2
23N2
30N2
49N2
41N2
53N2
09N2
13N2
28N3
29N3
23N3
30N3
49N3
41N3
53N3
09N3
13N3

FATIGUE QUESTIONNAIRE

ID	DATE

INSTRUCTIONS:
Please check (✓) "YES" or "NO" in the proper space, whether you have been experiencing the following DURING THE PAST WEEK.
Please answer each item.

Item No.	Description	YES	NO
1.	My head feels heavy		
2.	I am finding it difficult to think		
3.	I have a headache		
4.	My whole body feels tired		
5.	I am tired of talking		
6.	I feel stiff in the shoulders		
7.	My legs are tired		
8.	I feel nervous		
9.	I have a pain in my back		
10.	I feel like I have to yawn		
11.	I am unable to concentrate		
12.	I find it difficult to breathe		
13.	My mind feels dull or clouded		
14.	I am not interested in anything		
15.	I am thirsty		
16.	I feel drowsy		
17.	I have been forgetful		
18.	I feel dizzy		
19.	My eyes are tired and strained		
20.	I feel that I have no confidence		
21.	I feel that my eyelids are quivering		
22.	I feel very clumsy or stiff		
23.	I feel quite anxious		
24.	My arms or legs are shaky		
25.	I feel as if I am going to fall down		
26.	I feel unable to stand up straight		
27.	I feel quite sick		
28.	I want to lie down		
29.	I feel quite impatient		
30.	My voice feels quite sick		

From: Yositake, H. (1978). Ergonomics, 21, 231 - 233.

Circadian Rhythm Questionnaire

J. A. Horne and O. Ostberg

Instructions

1. Please read each question very carefully before answering.

2. Answer ALL questions.

3. Answer questions in numerical order.

4. Each question should be answered independently of the others. Do NOT go back and check your answers.

5. All questions have a selection of answers. For each question place a cross alongside ONE answer only. Some questions have a scale instead of a selection of answers. Place a cross at the appropriate point along the scale.

6. Please answer each question as honestly as possible. Both your answers and the results will be kept in strict confidence.

7. Please feel free to make any comments in the section provided below each question.

Please supply the information requested below.

ID: _____

1. Considering your own "feeling best" rhythm, at what time would you get up if you were free to plan your day?

| −+− | −+− | −+− | −+− | −+− | −+− | −+− |

a.m. 5 6 7 8 9 10 11 12

2. Considering your own "feeling best" rhythm, at what time would you go to bed if you were free to plan your evening?

| −+− | −+− | −+− | −+− | −+− | −+− | −+− |

p.m. 8 9 10 11 12 a.m. 1 2 3

3. If there is a specific time at which you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?

- Not at all dependent. . . . . ☐
- Slightly dependent . . . . . ☐
- Fairly dependent . . . . . ☐
- Very dependent . . . . . ☐

4. Assuming adequate environmental conditions, how easy do you find getting up in the morning?

- Not at all easy. . . . . ☐
- Not very easy . . . . . ☐
- Fairly easy . . . . . ☐
- Very easy . . . . . ☐

5. How alert do you feel during the first half hour after having woken in the morning?

- Not at all alert . . . . . ☐
- Slightly alert . . . . . ☐
- Fairly alert . . . . . ☐
- Very alert . . . . . ☐

6. How is your appetite during the first half hour after having woken in the morning?

- Very poor . . . . . ☐
- Fairly poor . . . . . ☐
- Fairly good . . . . . ☐
- Very good . . . . . ☐

7. During the first half hour after having woken in the morning, how tired do you feel?

- Very tired . . . . . ☐
- Fairly tired . . . . . ☐
- Fairly refreshed . . . . . ☐
- Very refreshed . . . . . ☐

8. When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?

- Seldom or never later . . . . ☐
- Less than one hour later . . ☐
- 1-2 hours later . . . . . ☐
- More than 2 hours later . . ☐

9. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him/her is between 7:00 - 8:00 a.m. Bearing in mind nothing else but your own "feeling best" rhythm, how do you think you would perform?

- Would be in good form ..... ☐
- Would be in reasonable form ..... ☐
- Would find it difficult ..... ☐
- Would find it very difficult ..... ☐

10. At what time in the evening do you feel tired and as a result in need of sleep?

	|	−	+	−	|	−	+	−	|	−	+	−	|	−	+	−	|	−	+	−	|	−	+	−	|
p.m.	8			9			10			11			12 a.m.			1			2			3				

11. You wish to be at peak performance for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day and considering only your own "feeling best" rhythm which ONE of the four testing times would you choose?

- 8:00 - 10:00 a.m. ..... ☐
- 11:00 a.m. - 1:00 p.m. .... ☐
- 3:00 - 5:00 p.m. ..... ☐
- 7:00 - 9:00 p.m. ..... ☐

12. If you went to bed at 11:00 p.m. at what level of tiredness would you be?

- Not at all tired ..... ☐
- A little tired ..... ☐
- Fairly Tired ..... ☐
- Very Tired ..... ☐

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which ONE of the following events are you most likely to experience?

- Will wake up at usual time and will NOT fall asleep . ☐
- Will wake up at usual time and will doze thereafter .. ☐
- Will wake up at usual time but will fall asleep again .. ☐
- Will NOT wake up until later than usual ..... ☐

14. One night you have to remain awake between 4:00 - 6:00 a.m. in order to carry out a night watch. You have no commitments the next day. Which ONE of the following alternatives will suit you best?

- Would NOT go to bed until watch was over ..... ☐
- Would take a nap before and sleep after ..... ☐
- Would get a good sleep before and a nap after .... ☐
- Would take ALL sleep before watch ..... ☐

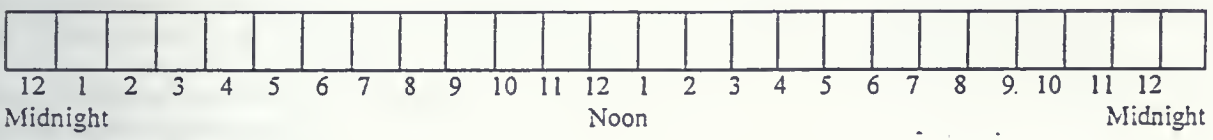
15. You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own "feeling best" rhythm which ONE of the following times would you choose?

- 8:00 - 10:00 a.m. ☐
- 11:00 a.m. - 1:00 p.m. ☐
- 3:00 - 5:00 p.m. ☐
- 7:00 - 9:00 p.m. ☐

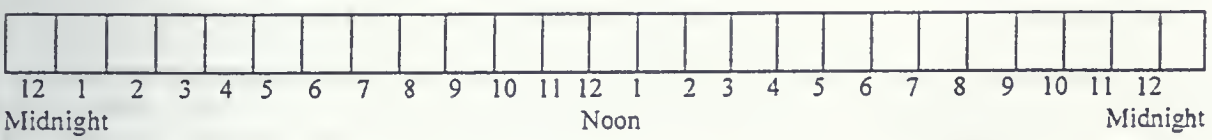
16. You have decided to engage in hard physical exercise. A friend suggests that you do this one hour twice a week and the best time for him/her is between 10:00 - 11:00 p.m. Bearing in mind nothing else but your own "feeling best" rhythm, how do you think you would perform?

- Would be in good form ☐
- Would be in reasonable form ☐
- Would find it difficult ☐
- Would find it very difficult ☐

17. Suppose that you can choose your own work hours. Assume that you worked a FIVE hour day (including breaks) and that your job was interesting and paid by results. Which FIVE consecutive hours would you select?



18. At what time of day do you think that you reach your "feeling best" peak?



19. One hears about "morning" and "evening" types of people. Which ONE of these types do you consider yourself to be?

- Definitely a "morning" type ☐
- Rather more a "morning" than an "evening" type ☐
- Rather more an "evening" than a "morning" type ☐
- Definitely and "evening" type ☐

Sleep and memory study 2003: Telephone Interview

ame:	Date:

I. DESCRIBE STUDY:

We are interested in the relationship between sleep and memory. In particular we are investigating how different types of learning change the quality of your sleep. You will be asked to come to the sleep for one afternoon for 2 hours from about 1:00 to 3:00 PM to fill out screening questionnaires so that we may determine your suitability for participating in this study. Following this, you will be given a sleep/activity diary and asked to keep track of your sleep and wake times, as well as your daily activities for 7 days. Following the 7 days of sleep/activity diary recording, you will be asked to return to the sleep laboratory from 9:00 PM to 8:00 AM for 3 consecutive nights to participate in a memory task and have your brain activity recorded overnight with electrodes placed on the scalp. One week following the second overnight night in the sleep laboratory, you will be asked to return for a 1 hour retest on the memory task. You will be given a 3 hour research credit for PSYC 1F90 (if enrolled) for completing the screening questionnaires and the sleep/activity diary. In addition, if suitable to participate, upon completion you will receive $75.00 ($25.00 per overnight) for your time and effort. Do you have any questions about the study at this point?

II. INCLUSION/EXCLUSION CRITERIA:

Based on information obtained from answers to the following question we will be able to determine how suitable you are to participate in this study. Do you mind taking some time to answer these questions?

1. Do you consider yourself a good sleeper? _____
   Are you right or left handed? _____
3. Is English your first language? _____
4. Are you a smoker or non-smoker? _____
5. How many caffeinated beverages do you have in a day? _____
6. What is your current course load/number of classes enrolled? _____
7. How many hours of studying do you do in an average week? _____
8. Are you currently engaged in any organized sports, or lessons that involve physical activity? _____
   Such as: piano lessons, driving lessons, any sports including going to the gym…
9. Do you have any hobbies that involve skills and strategy such as chess, or card games? _____

III. SLEEP:

1. What are your usual sleeping times (bedtime and wake time)? _____
2. Do you have difficulty falling asleep? _____
3. Do you wake up often in the night and are unable to return to sleep? _____
4. Would you describe yourself as excessively tired during the day? _____
   during lectures? While watching tv? While driving, or during physical activity?

IV. HEALTH:

1. Are you presently in good health? _____
2. Any prescription or non-prescription medication? _____
3. Any history of depression? _____
4. Any history of head injury (accident, stroke, loss of consciousness)? _____
5. Any history of chronic pain? _____

BROCK UNIVERISTY DEPARTMENT OF PSYCHOLOGY
Informed Consent Agreement and Letter of Information

Title of study: "The role of sleep in memory consolidation".
Researcher: Stuart Fogel
Office: Brock University Sleep Research Laboratory, MC B417
Telephone: (905) 688-5550 ext. 3795
Email: sf02sr@brocku.ca

Thesis Supervisor: Dr. Kimberly Cote
Telephone: (905) 688-5550 ext 4806

Name of participant: (Please print your name) _____

I understand that I have agreed to participate in a research study that will investigate the relationship between sleep and memory. Past research in the area of sleep and memory has shown that sleep deprivation impairs recall on a memory task. In the present study, we hope to determine how sleep changes as a result of new learning.

As a participant in this study, I will be asked to be in the sleep laboratory at Brock University for one, 2-hour testing session, one week prior to overnight sleep recording, and keep a sleep/activity of your sleep patterns and daily activities for seven days thereafter. In addition, I will be asked to take part in 3 consecutive overnight sessions, each 11 hours in length from 9:00 PM to 8:00 AM. During the overnight sessions in the sleep laboratory, I understand that my brain activity will be monitored and recorded.

I will have the opportunity to learn about neuroscience research in the lab, and benefit from helping to forward knowledge about the brain mechanisms responsible for formation of new memories. In addition, I will learn about how research is conducted in a sleep research facility, and how brain activity is monitored during sleep.

This study will help forward the understanding of the brain mechanisms involved in the formation of new memories. It is expected that this study may identify the specific types of sleep necessary for the efficient formation of particular types of memory.

My name will not appear in any report, publication or presentation resulting from this study. The data, with identifying information removed, will be retained indefinitely and will be securely stored in a locked office in the research laboratory. These data will be used solely for a Masters Thesis. Findings will be reported in the thesis dissertation and manuscript.

If enrolled in PSYC 1F90, I understand that I will be given 3 hours research credit toward Psychology 1F90 and for preliminary screening and for filling out questionnaires. I understand that if I meet the inclusion criteria, and I choose to continue to participate in the remainder of the experiment including the overnight sleep recording I will receive $25.00 per night (in laboratory) for my participation. Participants may experience temporary redness of the skin and or dry irritated skin, due to application of skin electrodes. If skin irritation occurs, it dissipates shortly after the removal of the electrodes and may be alleviated by applying skin moisturizer. All procedures should not cause any physical or psychological stress.

I understand that my participation in this study is voluntary, that I may withdraw from the study at any time and for any reason without penalty, and that I may ask questions at any time.

I understand that there is no obligation to answer any question/participate in any aspect of this project that I consider invasive, offensive or inappropriate.

I understand that all personal data will be kept strictly confidential and that all information will be coded so that my name is not associated with my answers. I understand that only the researchers named above will have access to the data.

My signature below indicates that I have read and understood the procedures of the study and that I give my free and informed consent to participate.

Participant's signature _____ Date _____

This project has been reviewed by, and received ethics clearance through, the Office of Research Ethics Board. (REB file #03-002)

In the event you have any question or concerns about your participation in this study, please contact the Research Ethics Officer at 905-688-5550, Ext. 3035.

If you have any questions or concerns about your participation in this study, you may contact Stuart Fogel or Dr. Kimberly Cote.

Thank you for your help! Please take one copy of this form with you for further reference.

I have fully explained the procedures of this study to the above volunteer.

Researcher's signature: _____ Date _____

SLEEP - WAKE QUESTIONNAIRE

ID	DATE (dd/mm/yy)	HEIGHT	WEIGHT	SEX

INSTRUCTIONS

Read each of the questions carefully and select a scale number that best describes HOW OFTEN YOU HAVE HAD THESE DURING THE PAST **2 MONTHS**. Place the number in the block immediately to the right of the item. Do not skip any item and print your numbers clearly. Make sure that you have answered all the questions.

SCALE

0 Never	2 Sometimes	4 Always
1 Rarely	3 Often	5 N/A

EXAMPLE

How often do you awaken more than 5 times at night?

Answer: 3

(This means that you **often** awaken more than 5 times at night)

1 Before going to sleep how often do you engage in the following activities:

a) Read . . . . . ☐
b) Smoke . . . . . ☐
c) Eat a snack . . . . . ☐
d) Watch TV . . . . . ☐
e) Drink tea, coffee, cola . . . . . ☐
f) Drink water, soft drinks . . . . . ☐
g) Listen to music or radio . . . . . ☐
h) Take sleeping pills or tranquillizers . . . . . ☐
i) Shower or bath . . . . . ☐
j) Exercise or take short walks . . . . . ☐
k) Relaxation exercises (Meditation, Prayer) . . . ☐
l) Engage in other activities . . . . . ☐

2 How often does it take you more than 30 minutes to fall asleep? . . . . . ☐

3 How often are you unable to sleep at all? . . . . ☐

4 Before falling asleep, how often do you experience any of the following:

a) Coughing, breathing difficulties, suffocation . ☐
b) Feeling hot and sweaty . . . . . ☐
c) Headaches . . . . . ☐
d) Confusion/disorientation (do not know where you are) . . . . . ☐
e) Tension and worry . . . . . ☐
f) Unpleasant thoughts . . . . . ☐
g) Aches or pains in: limbs . . . . . ☐
neck . . . . . ☐
back . . . . . ☐
chest . . . . . ☐
abdomen . . . . . ☐
h) A need to move your legs because of unpleasant sensations in them . . . . . ☐
i) Sudden jerking movements of your arms and legs . . . . . ☐
j) Unable to move arms or legs . . . . . ☐
k) Unable to stop thinking about recent or past events . . . . . ☐
l) Frightening or strange hallucinations . . . . . ☐
m) Other (specify) . . . . . ☐

5 How often do you awaken more than 3 times at night? . . . . . ☐

6 If you awaken at night, how often do you stay awake more than 30 minutes before you go to sleep again? . . . . . ☐

7 If you awaken at night, how often do these awakenings happen during:

a) the first third of the night . . . . . ☐
b) the second third of the night . . . . . ☐
c) the last third of the night . . . . . ☐

Scale: 0 = Never; 1 = Rarely; 2 = Sometimes; 3 = Often; 4 = Always; 5 = N/A

8 How often are your awakenings during the night due to:

a) External noises (telephone, baby crying noisy traffic) ☐
b) Nightmares or unpleasant dreams ☐
c) Aches and pains in different parts of the body (specify) ☐
d) Coughing, choking, breathing difficulties ☐
e) Sudden jerking movements of arms and legs ☐
f) Need to urinate ☐
g) Heartburn ☐
h) Headache ☐
i) Other (specify) ☐

9 During your sleep at night, how often have you noticed or have you been told that you do any of the following?

a) Snore ☐
b) Turn your head from side to side ☐
c) Move your arms or legs or kick ☐
d) Talk during your sleep ☐
e) Walk during your sleep ☐
f) Scream or shout ☐
g) Grind your teeth ☐
h) Cough ☐
i) Suffer from interrupted breathing ☐
j) Bed wetting ☐

10 How often is your sleep:

a) Light ☐
b) Deep ☐

11 How often do you have disturbing (bad) dreams during sleep? ☐

12 When you wake up from these bad dreams, how often do you have the following?

a) Feel relieved ☐
b) Feel frightened ☐
c) Have no emotion at all ☐
d) Feel heart pounding ☐
e) Breathe heavily ☐
f) Choke ☐
g) Feel pressure on your chest ☐
h) Not able to move ☐
i) Feel restless ☐
j) Perspire ☐
k) Scream ☐
l) Wet your bed ☐
m) Immediately fall asleep ☐

13 How often do you awaken in the morning feeling:

a) Good mood ☐
b) Bad mood ☐
c) Refreshed ☐
d) Physically tired ☐
e) Headache ☐
f) Muscle stiffness or pain in: limbs ☐ neck ☐ back ☐ chest ☐ abdomen ☐

14 How often do you nap during the day:

a) On work/school days? ☐
b) On weekends and holidays? ☐

15 How often do you feel refreshed after a daytime nap? ☐

16 How often do you fall asleep during the following situations?

a) While travelling (car, train, etc.) ☐
b) In the movies or theatre ☐
c) During talks or lectures ☐
d) While watching TV ☐
e) During social situations ☐
f) While reading ☐
g) During work ☐
h) While driving a car ☐
i) While eating ☐
j) During other activities (specify) ☐

17 How often do you stop an activity because of an irresistible need to sleep? ☐

Scale: 0 = Never; 1 = Rarely; 2 = Sometimes; 3 = Often; 4 = Always; 5 = N/A

18 During the day, how often do you:

a) Feel refreshed and energetic ..... ☐
b) Feel physically exhausted and listless ..... ☐
c) Yawn ..... ☐
d) Have problems at work/school due to sleepiness or naps ..... ☐
e) Have attacks of sudden muscle weakness or falling ..... ☐
f) Have automatic activity (i.e., driving or walking without recalling where you are) .... ☐
g) Feel faint or lose consciousness ..... ☐
h) Feel dizzy or unsteady ..... ☐
i) Have unusual sensation (numbness, tingling) in arms and legs ..... ☐
j) Have headaches ..... ☐
k) Have pain or discomfort in: limbs ..... ☐
neck ..... ☐
back ..... ☐
chest ..... ☐
abdomen .... ☐

19 How often do you have to work on shifts? .. ☐

20 How often do you work on the:

a) Day shift? ..... ☐
b) Evening shift? ..... ☐
c) Night shift? ..... ☐

21 How often does your work require you:

a) to stay awake most of the night? ..... ☐
b) to travel from one time zone to another? .... ☐

22 How often during your work are you exposed to:

a) Continuous noises? ..... ☐
b) Monotonous activity? ..... ☐
c) Social isolation? ..... ☐
d) Pressures to increase your work output? .... ☐

23 How often have you used medications for the following purposes?

a) to relieve pain (e.g. aspirin). Specify ..... ☐
b) to relieve heartburn or indigestion (e.g. Antacids). Specify ..... ☐
c) to diminish nervousness (e.g. Tranquillizer) Specify ..... ☐
d) to relieve depression (e.g. antidepressant) Specify ..... ☐
e) to help you sleep better. Specify ..... ☐
f) to keep you awake during the day. Specify ... ☐
g) caffeine tablets ..... ☐
h) against allergy (e.g. anithistamines). Specify ☐
i) against asthma (e.g. aminophylline). Specify . ☐
j) to prevent convulsions (e.g. dilantin). Specify ☐
k) to treat heart problems. Specify ..... ☐
l) to treat respiratory problems. Specify ..... ☐
m) to treat high blood pressure. Specify ..... ☐
n) hormones. Specify ..... ☐
o) to treat Parkinsonism. Specify ..... ☐
p) to reduce weight. Specify ..... ☐
q) other types of medicines. Specify ..... ☐
(1) _____
(2) _____
(3) _____

24 How often have you used:

a) Marijuana/hash? ..... ☐
b) Cocaine/crack? ..... ☐
c) L.S.D., mescaline, ecstasy? ..... ☐
d) Stimulants (speed drugs, uppers, mood elevators, ephedrine)? ..... ☐
e) Narcotics (morphine, heroin, opium)? ..... ☐
f) Other. Specify? ..... ☐

SLEEP - WAKE QUESTIONNAIRE – Part II

INSTRUCTIONS

The following are statements that describe some measurable aspects of your experience. Read each statement carefully and put in the appropriate box the nearest number that describes your experience.

If the statement does not apply to you, put "N/A" on the appropriate line.

1. During work/school days, I usually sleep _____ hours.

2. During weekends and holidays, I usually sleep _____ hours.

3. If I nap, they usually last _____ minutes each .

4. During the past 6 months, I have had _____ nightmares each week.

5. During the past 3 years because of sleepiness : (a) I had _____ work accidents during day time.
   (b) I had _____ work accidents during night time.
   (c) I had _____ accidents during day time.
   (d) I had _____ car accidents during night time.

6. During the past month, I had to change: (a) from morning shift to night shift _____ times.
   (b) from night shift to morning shift _____ times.
   (c) from evening shift to night shift _____ times.
   (d) from night shift to evening shift _____ times.
   (e) from morning shift to evening shift _____ times.
   (f) from evening shift to morning shift _____ times.

7. Each day I usually drink: _____ (a) cups of caffeinated coffee.
   _____ (b) cups of regular tea
   _____ (c) cups of herbal tea, Specify types: _____

8. Each day I usually take: _____ (a) vitamins; Specify _____
   _____ (b) herbal remedies; Specify _____

9. Each day I usually smoke: _____ (a) cigarettes.
   _____ (b) other; Specify _____

10. Each week I usually drink: _____ (a) glasses of cola.
    _____ (b) glasses of wine.
    _____ (c) bottles of beer.
    _____ (d) ounces of liquor; Specify _____
    _____ (e) ounces of other liquor; Specify _____

FAMILY SLEEP HISTORY

Please check (✓) in the proper space if any of the following items apply to a member of your family.

		Son	Daughter	Brother	Sister	Mother	Father	Other
1	Sleep walking							
2	Screaming during sleep							
3	Very loud snoring in sleep							
4	Daytime sleepiness							
5	Other sleep problems (specify)							
	a)_____							
	b)_____							
	c)_____							
6	Chronic fatigue							
7	Epilepsy							
8	Mental illness							
9	Psychiatric treatment							
10	Death during sleep							
	Chronic diseases:							
11	Cancer							
12	Heart diseases							
13	Rheumatoid arthritis							
14	Diabetes mellitus							
15	Other chronic disease							

HEALTH QUESTIONNAIRE

Please check (✓) in the proper space only the items in the following list that apply to you.

		During the Past Year A	More Than A Year Ago B
1	Diabetes		
2	Thyroid disorders		
3	Epilepsy		
4	Psychiatric illness		
5	Psychiatric treatment		
6	Neurologic disease		
7	Kidney disease		
8	Peptic ulcer, gastritis		
9	Intestinal disease (colitis)		
10	Liver disease		
11	High blood pressure		
12	Heart disease		
13	Headache		
14	Arthritis		
15	Back pain		
16	Obesity		
17	Asthma		
18	Pneumonia		
19	Enlarged tonsils, adenoids		
20	Repeated throat infections		
21	Chronic sinusitis		
22	Deviated Nasal Septum		
23	Other health problems (Specify)		
	Hospitalization:		
24	1 or 2 times		
25	3 or 4 times		
26	More than 4 times		
27	Surgery on mouth and/or nose (Specify)		
	For Women Only:		
28	Irregular menstrual periods		
29	Use of birth control pills		
30	Problems associated with menopause		

Brock University Sleep Research Laboratory
Sleep and Activity Diary

Name:

Instructions:

- Please leave the Sleep and Activity Diary by your bedside and fill in daily activities each evening and sleep time and duration each morning.
- An example of how the log should be marked is given at the bottom of this page.

Suggested Guidelines:

- For the seven days preceding the nights in the lab, please try:
  - To keep regular sleep times and wake times (for example: 11PM to 7AM).
  - Do not drink *excessive* amounts of alcoholic beverages.
  - To eat and exercise at your regular times.
- Do not drink any alcoholic beverages the night before the first night in the lab.
- Do not drink any alcoholic beverages on days before the nights in the lab.
- Do not drink more than one caffeinated beverage (if applicable) on days before the nights in the lab.
- Please wake up by 8:00 AM the morning of the first night in the lab.
- Please do not nap before nights in the lab.

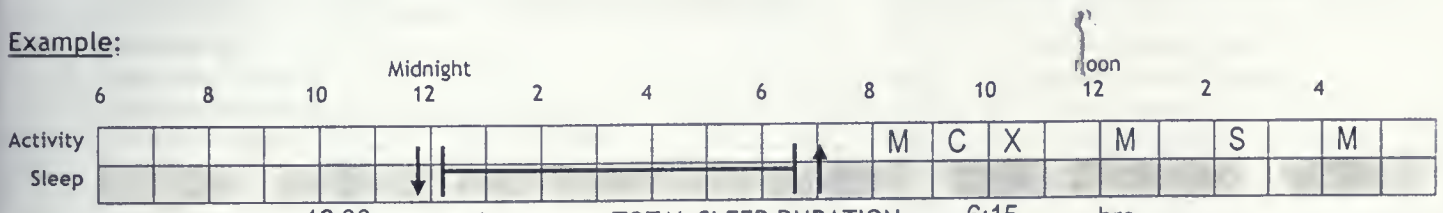
Contact Information

If you need to contact Stuart about the study, please email me at sf02sr@brocku.ca, or call the Brock sleep lab at (905) 688-5550 ext. 3795.

The appropriate activities that should be logged include:

ACTIVITIES	
C	Any caffeinated drinks including coffee, tea, cola, etc.
A	Any alcoholic beverages
M	Meals
X	Exercise/Physical activity (please specify in comments)
T	Use of toilet during sleep time
S	Studying (please specify type of work in comments)
SLEEP (INCLUDING NAPS)	
↓	An arrow "down" roughly marks the time you went to bed
↑	An arrow "up" roughly marks the time you got out of bed
|	Mark with lines the time you began and ended your sleep
—	By joining these two lines you're indicating a sleep period

Example:



LIGHTS OUT ____12:00____ am/pm TOTAL SLEEP DURATION ____6:15_____ hrs

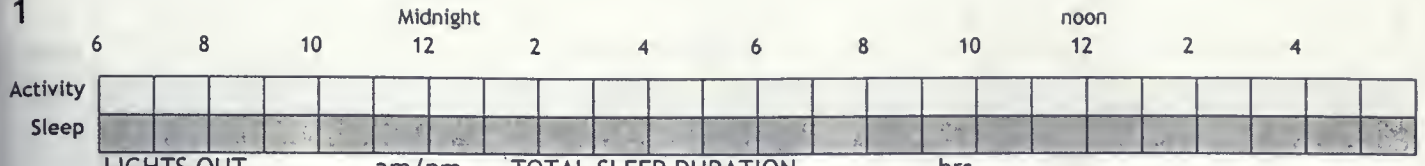
Comments:_____felt tired most of the day_____

Describe Exercise:_____Jogging 10 AM (15 minutes)_____

Other activities:_____Played video games for 30 minutes, played guitar for 1 hour_____

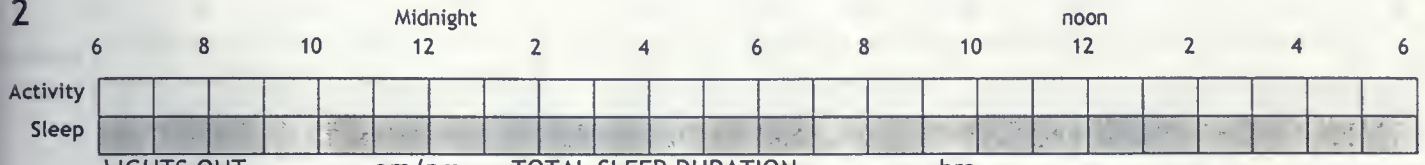
Hours of studying:__2___ Type of work:_____biology (1hr),_Calculus (1hr)_____

1



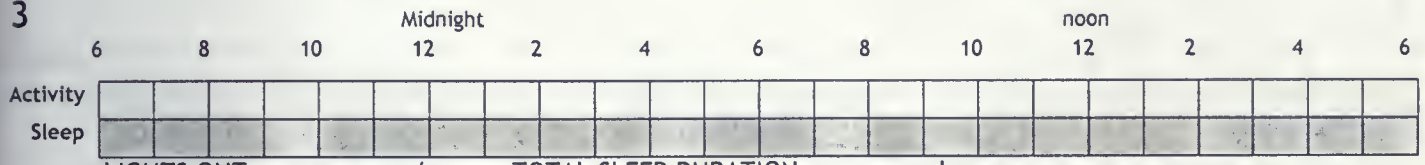
LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

2



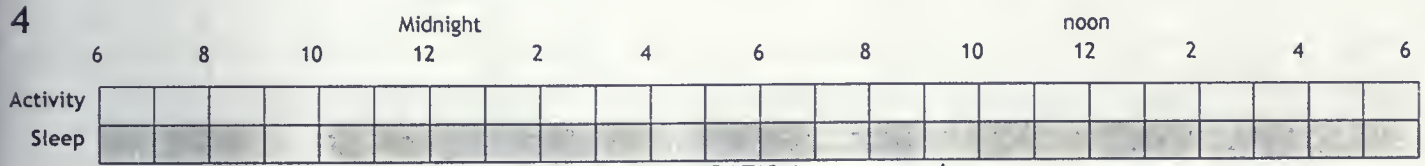
LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

3



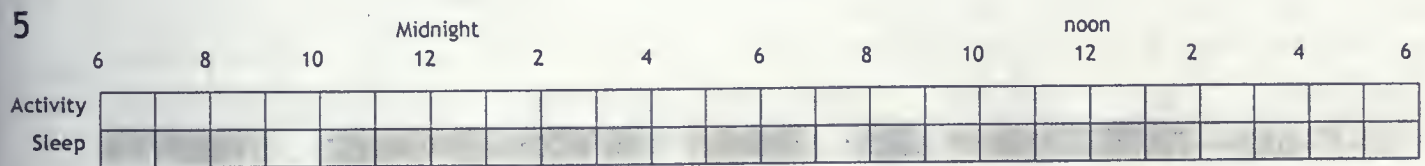
LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

4



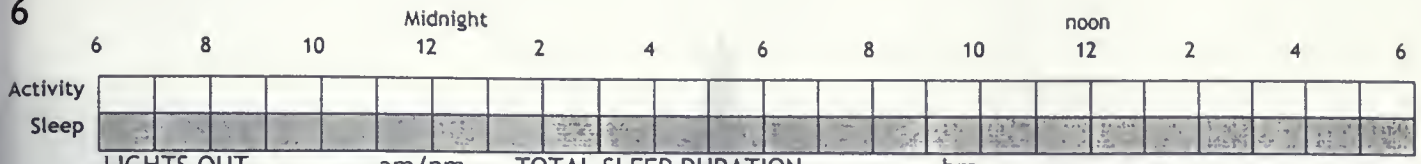
LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

5



LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

6



LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

7

LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

8

LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

9

LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

10

LIGHTS OUT _____ am/pm TOTAL SLEEP DURATION _____ hrs
Comments:_____
Describe Exercise:_____
Other activities:_____
Hours of studying:_____ Type of Work:_____

PARTICIPANT FEEDBACK INFORMATION

'tle of study: "The role of sleep in memory consolidation".

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There is a growing body of research that indicates that sleep is important for the efficient formation of new memories. Various stages of sleep are thought to be important for the strengthening of particular types of memory. The function of stage 2 and REM sleep remains to be determined. Previous research has found that stage 2 sleep deprivation impairs memory for motor skills, but not more cognitively complex types of memory. Rapid eye movement (REM) sleep deprivation impairs cognitively complex memory, but not motor skills memory. The present study is designed to investigate how new learning affects stage 2 sleep and REM sleep in normally rested individuals.

The study that you have participated in examined the effect of new learning on the brain wave activity of REM and Stage 2 sleep. You were in one of four experimental groups including those that learned a motor skills task, a complex logic task, a declarative (verbal) task or a control task (no sleep-dependent learning). The EEG of these groups will be compared to determine how new learning affects sleep patterns and EEG waveforms. We predicted that motor skills learning would increase a naturally occurring brainwave called the sleep spindle. The sleep spindle is thought of as one of the brain mechanisms for encoding newly learned material into long-term memories. In addition, we predicted that complex logic learning would increase the number of rapid eye movements during REM sleep. Rapid eye movements have also been linked to the formation of new learning. 'inally, it was hypothesized that declarative learning would change the type of brain activity during REM sleep.

This research is intended to extend previous findings, and further knowledge about how memories are formed and strengthened during sleep. This research has important practical applications in terms of the need for sleep in order to efficiently learn new material that involves the refinement of motor skills, procedures, and verbal knowledge.

Thank you for participating in this research. We hope that it was an educational and rewarding experience for you. The final results of this study will be available within a year. If you would like a copy of the written results, they are available upon request. If you would like to know more about our research, please contact Stuart Fogel, or Dr. Kimberly Cote in the Psychology Department by email or phone.

This study has been reviewed by, and received ethics clearance through, the Office of Research Ethics, Brock University. For more information please contact the Research Ethics Officer at 905-688-5550 x 3035. (REB file #03-002).