THE EFFECT OF SLEEP ON THE CONSOLIDATION OF DECLARATIVE AND PROCEDURAL MEMORY

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy

The City University of New York

2007

This manuscript has been read and accepted for the
Graduate Faculty in Psychology in satisfaction of the
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Abstract

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The following three studies set out to examine the effect of sleep on memory with an emphasis on the effect of NREM sleep on declarative memory. The design of each study is largely behavioral with attempts to relate memory performance to relevant EEG correlates when possible. The first study is a replication of a study by Plihal & Born (1997) using a daytime nap design that eliminates the effects of sleep deprivation and assesses memory after a sleep episode containing only NREM sleep. We were able to replicate their findings by showing that NREM sleep facilitates processing of declarative memory (paired associates) while having no effect on procedural memory (mirror tracing). The second study expands on these finding by examining the effect of a daytime nap on three declarative memory tasks that either carry a strong semantic loading (unrelated paired associates) or lack semantic value (spatial maze learning and complex figure drawing). We found a sleep-dependent facilitation of performance for the paired associates task, but not for the non-semantic tasks. However, for all three tasks there was a marked sleep-dependent effect if subjects strongly encoded the information prior to sleep, suggesting that sleep may better process well-learned information while having little effect on weakly encoded information. The last study employs a nocturnal sleep

design to assess the effect of sleep duration (3.5 v. 7.5 hours of sleep) on performance on a declarative (related paired associates) and a motor (number sequence learning) learning task. The subject variable intelligence is also assessed as a potential modulator of the effect of sleep on memory. We found that improvement in performance on both tasks after sleep was almost identical for both sleep groups and, surprisingly, this improvement is identical to improvements shown to occur following a short daytime nap (Tucker et al., 2006; Nishida & Walker, 2006). It was shown that intelligence was positively correlated with encoding and retest performance, but not with improvement following sleep, suggesting that while more intelligent subjects demonstrate greater encoding facility, encoding strength under these conditions does not lead to enhanced sleep-related memory performance.

Acknowledgements

The following researchers played critical roles in the completion of the work described here: Dr. William Fishbein, Erin Wamsley, and Yasutaka Hirota. I would also like to thank all members of the research team at the Laboratory of Cognitive Neuroscience and Sleep: Hiuyan Lau, Annie Chaklader, Sara Alger, Rafael DeJesus, and Jason Birnbaum. The following research represents a collaborative effort that would not have been possible without the contribution of these individuals.

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I

General Introduction

The Sleep Effect

For almost 100 years researchers have attempted to explain a phenomenon known as the "sleep effect", the consistent observation that sleep facilitates recall of information more than equivalent periods of wakefulness (Ekstrand, Barrett, West, & Maier, 1977). Over the decades interest in this phenomenon has waxed and waned in step with evolving conceptions of the function of sleep, with the most intense research efforts, on every level of analysis, occurring in the past 10-15 years. The basis for this resurgent interest has to do largely with our growing understanding of the dynamic, complex nature of sleep as well as the physiological mechanisms and time course of memory consolidation. Not surprisingly, this interest is also a result of the shedding of long-held assumptions regarding the nature of the sleeping brain. For example, a handful of findings from the early 1970s strongly suggested that slow wave sleep (SWS) benefits recall of paired associates (Yaroush, Sullivan, & Ekstrand, 1971; Barrett & Ekstrand, 1972; Fowler, Sullivan, & Ekstrand, 1973). However, these investigators based their interpretation on the idea that SWS (a time of electroencephalographic (EEG) quiescence) functioned merely to slow the decay of the memory trace; the possibility that an active mnemonic process could be taking place during this sleep state was not entertained. This general interpretation of the sleep effect, in various incarnations, is expressed in the sporadic studies conducted prior to the 1960s, which date back to the turn of the century (Jenkins & Dallenbach, 1924). Rapid eye movement (REM) sleep, on the other hand, was thought initially to contribute to memory processing because the cognitive correlate of REM sleep

(i.e., dreaming) was widely believed to represent a replay of previous waking experience or, to use the Freudian term, "day residue." These early conceptions of sleep-related memory processing were necessarily superficial, as scientists lacked the technologies that would allow for a deeper physiological analysis of the sleeping brain. Therefore, explanations of the sleep effect were largely confined to speculative arguments based on behavioral outcomes.

Although experimental research on the relationship between sleep and memory dates back almost a century, a concerted effort to understand this relationship is not observed until the mid 1960s, due in large part to the groundbreaking identification of the EEG correlates of REM sleep by Eugene Aserinsky and Nathaniel Kleitman (Aserinsky & Kleitman, 1953). For well over a decade, from the mid 1960s through the mid 1970s, intensive investigation of the effect of sleep (especially REM sleep) on memory performance yielded a number of significant findings (for review see Fishbein & Gutwein (1977)). However, this line of research virtually dissolved in the mid 1970s in the face of growing methodological concerns and perceived inconsistencies between human and animal findings. Indeed, during the 1980s one sees nearly an equivalent number of critical reviews on the topic as positive empirical findings. This diminished interest in sleep and memory, however, cannot be reduced to a simple balance sheet analysis of positive and negative findings. An equally incapacitating problem was that, without a proper understanding of the neurophysiological basis of memory processing, scientists were simply unable to adequately characterize the sleep-specific neurophysiology capable of promoting memory consolidation.

It was not until the synthesis of a number of loosely related findings from multiple neuroscientific perspectives that interest in the topic rekindled. The first breakthrough was the identification by Gyorgy Buzsaki of the hippocampal sharp wave/ripple (SPW-R) complex, which was found to be most strongly expressed during SWS (Buzsaki, 1986). Because the hippocampus is intimately linked to the encoding and consolidation of semantic and episodic (i.e., declarative) information, the SPW-R event began to be viewed as a candidate neuronal correlate of memory processing during sleep. Following this discovery, a number of interesting findings further elucidated the potential functionality of the SPW-R. For example, the ripple component of the SPW-R, a highly synchronous, high frequency (~200Hz) discharge of hippocampal CA1 pyramidal neurons, was shown to propagate out through deep layers of entorhinal cortex primarily during SWS, suggesting that these events may potentiate neocortical targets during the sleep that follows waking information processing (Chrobak & Buzsaki, 1994). The ripple is also endowed with properties capable of inducing long term potentiation (LTP), which is by far the most promising physiological correlate of memory. In hippocampal slice preparations Buzsaki et al. (1989) showed that induction of SPW-R events led to LTP in neurons of the Schaffer collateral system.

While these findings suggest a role for SPW-R events in sleep-related memory consolidation, it should be noted that a direct link between these events and memory enhancement is lacking:

> "Though the model is firmly grounded in current knowledge of neuroanatomy and physiology, it remains long on speculation and short on data." (Buzsaki, 1998)

However, experimental support linking behavior with physiology has come from recent findings showing that learning of a radial arm maze task (a declarative memory task) in rats leads to a sharp increase in ripple expression during subsequent NREM sleep (Ramadan, Yeshenko, & Sara, 2006), which represents a positive first step in establishing the mnemonic value of the SPW-R complex.

The second major breakthrough was the discovery by Jonathan Winson and Gus Pavlides that individual hippocampal cells that fired only in very specific places in the environment (hence the name "place cells") tended to fire at similar rates during subsequent sleep (Pavlides & Winson, 1989), suggesting that pre-sleep experience might be "replayed" during subsequent sleep. This finding has been corroborated several times since then by Bruce McNaughton, Matthew Wilson, and colleagues using multicellular recording techniques, providing strong evidence that spatiotemporal neuronal firing patterns occurring in the hippocampus during pre-sleep experience are replayed in a highly correlated manner during sleep (Wilson & McNaughton, 1994; Louie & Wilson, 2001; Lee & Wilson, 2002).

Lastly, the expansive literature on the role of acetylcholine (ACh) for memory processing has led to a better understanding of how wake and sleep contribute to the different stages of memory processing. The central hypothesis, elaborated most convincingly in the work of Michael Hasselmo, postulates that information encoding is best facilitated during periods of elevated ACh (i.e., during wakefulness), while consolidation should occur optimally during periods of low ACh (i.e., during SWS) (Hasselmo, 1999; Hasselmo & McGaughy, 2004). This cholinergic modulation of memory consolidation during wake and sleep has been elegantly demonstrated by Jan

Born and colleagues. In one study, following learning of paired associates, ACh was elevated in one group of subjects during the first few hours of sleep through the administration of physostigmine (Gais & Born, 2004). Paired associate recall was impaired in this group compared to subjects that received a placebo, which strongly supports the idea that the low levels of ACh observed during SWS are necessary for memory consolidation. Conversely, suppression of waking ACh levels by the ACh antagonist scopolamine 30 minutes following encoding of paired associates (mimicking the low ACh levels observed during SWS), resulted in superior paired associate recall later in the day compared to subjects that received a placebo (Rasch, Born, & Gais, 2006).

At the behavioral level, two pioneering studies are worthy of mention. The first is the study by Avi Karni and Dov Sagi showing that performance on a visual discrimination task (VDT) is dependent on REM sleep (Karni et al., 1994). The VDT is a perceptual learning task that requires subjects to identify the orientation (vertical or horizontal) of three diagonal lines that are situated against an array of horizontal lines. The array is presented for progressively decreasing amounts of time (starting from 400ms) until the subject obtains 80% identification accuracy. Because processing of the VDT is highly localized to retinotopically specific regions of the visual cortex, the location of the diagonal lines can be moved to different quadrants of the array allowing for multiple novel learning trials for each subject. In their now classic experiment, Karni et al. (1994) had subjects learn the VDT followed by two uninterrupted nights of sleep. On the third and fourth night subjects were deprived of either REM sleep or SWS. The study revealed that only those subjects that were REM deprived showed impaired

performance, while SWS deprivation resulted in performance that was comparable to the uninterrupted sleep group. This was the first experimental human study to show a powerful REM-specific sleep effect, which led to a serious reconsideration of the sleep-dependent memory consolidation hypothesis.

The second influential study built on the early research findings of Bruce Ekstrand and colleagues showing that a period of sleep rich in SWS during the first half of the night facilitates recall of paired associates, while REM-rich sleep, which predominates during the second half of the night, confers reduced memory enhancement (Yaroush et al., 1971; Barrett & Ekstrand, 1972). Starting from a basic knowledge of memory systems, Plihal & Born (1997) adopted the Ekstrand design to evaluate the effects of SWS and REM sleep on declarative (hippocampus-dependent) and procedural (hippocampus-independent) tasks. Using a standard declarative (paired associates) task and a widely used procedural task (mirror tracing), Plihal and Born were able to show that early sleep facilitates declarative memory recall compared to performance after late sleep or equal amounts of wakefulness, and that procedural memory performance benefited most after late sleep compared to early sleep or wakefulness. This elegant demonstration of the dissociation of sleep stage and memory domain finally integrated existing knowledge of memory systems with the study of sleep-related memory processing, and suggested that physiological properties specific to SWS and REM sleep might underlie the differences in processing of different types of memory.

Building on the aforementioned findings, a well-formulated, brain-based perspective of sleep-related memory consolidation has emerged that provides a stable foundation, from which long-standing questions about the effect of sleep on memory can

begin to be answered. For example, now that declarative memory processing has been linked to hippocampal processes acting during NREM sleep, we can begin to elaborate on the role of this type of sleep for memory processing. If NREM sleep is important for memory consolidation, across what range of declarative tasks is it beneficial? Does it only benefit performance on traditional verbal memory tasks (paired associates and story learning) that have a strong semantic component, or does it benefit a broader range of non-verbal/non-semantic declarative memory tasks? Also missing in the literature is a thorough analysis of the amount of sleep needed to consolidate different types of memories. Recent studies have shown that short durations of sleep may be enough to enhance consolidation (Mednick, et al. 2003), suggesting that longer periods of sleep may simply serve to maintain those early gains. Lastly, we know very little about how individual differences impact on the sleep-related consolidation of memories. Understanding the modulating effects of subject variables (e.g., general intelligence) may help us better understand the conditions under which sleep more powerfully amplifies the consolidation process.

The following three studies represent an attempt to address these broad issues by examining performance after various periods and types of sleep using a range of memory tasks, with an emphasis on the relationship between NREM sleep and declarative memory processing. The memory benefits that follow sleep are linked, when possible, to their EEG correlates as well as relevant subject variables. The reader should note that while these three studies begin to answer relevant questions regarding the nature of sleep-related memory consolidation, they also raise a number of interesting questions that should be addressed in future research.

II

A Daytime Nap Containing Solely Non-REM Sleep Enhances Declarative but not Procedural Memory

Introduction

After more than 40 years of research it has become well known that performance on procedural memory tasks (e.g., visual discrimination, mirror tracing, etc.) is enhanced following periods of sleep containing rapid eye movement (REM) sleep, and that performance on these tasks is impaired following REM sleep deprivation (Karni et al., 1994; Stickgold et al., 2000; Mednick, Nakayama, & Stickgold, 2003; Fishbein & Gutwein, 1977; Smith, 2001). In humans, the evidence is much weaker that REM sleep promotes processing of declarative (hippocampus dependent) memories (e.g., fact-based information and memory for personal events), which has led some to abandon the idea that sleep-specific physiological processes play any role at all in the processing of declarative memories (Vertes, 2004; Siegel, 2001). However, a strong case has recently been made for the involvement of non-REM (NREM) sleep (Stages 2, 3, & 4), and in particular slow wave sleep (SWS; Stages 3 & 4), in the processing of declarative memories. A small number of behavioral studies have shown that periods of sleep dominated by SWS enhance declarative memory recall but do not affect performance on procedural memory tasks (Plihal & Born, 1997, 1999a). These studies are complemented by compelling neurophysiological hypotheses outlining the potential mnemonic mechanisms of SWS-specific declarative (hippocampus-related) memory processing (Buzsaki, 1989, 1998; Hasselmo, 1999; Sejnowski & Destexhe, 2000). However, despite

these promising findings, there are some outstanding issues to be addressed to more firmly establish this relationship, including the use of research designs that isolate the effects of NREM sleep independent of REM sleep, and the development of research designs that avoid sleep deprivation.

Of the handful of behavioral research designs examining the benefits of sleep for memory processing in humans, the early/late sleep paradigm has consistently revealed a dissociation between memory system tested (procedural v. declarative) and stage of sleep (SWS v. REM) (Plihal & Born, 1997, 1999a; Yaroush et al., 1971; Barrett & Ekstrand, 1972). By comparing performance after the first 3-4 hours of nocturnal sleep, which is dominated by SWS, with performance after the last 3-4 hours of sleep, during which REM sleep predominates, it is possible to evaluate the relative contribution of these two stages of sleep to processing of different types of memory. It should be noted that the amount of stage 2 sleep is nearly equal in the early and late halves of the night. Using this design, it has been shown that early sleep facilitates recall of declarative information (paired associates learning and spatial memory), and that late sleep benefits performance on procedural memory tasks (mirror tracing) (Plihal & Born, 1997). These studies also demonstrate that subjects that sleep, either early or late in the sleep period, perform better than their counterparts that stay awake during these intervals. This early/late sleep design has been used to assess the relationship between sleep stage and emotional memory (Wagner, Gais, & Born, 2001), sensory memory (Gais, Plihal, Wagner, & Born, 2000), and recognition memory (Drosopoulos, Wagner, & Born, 2005).

While the findings of the above mentioned studies provide encouraging evidence that SWS facilitates declarative memory processing, several unresolved issues remain.

Though it is true that the first half of the sleep period contains increased amounts of SWS, it still contains a substantial amount of REM sleep. It is common for a normal sleeper to cycle through two NREM/REM periods during this 3-hour sleep interval, which leaves open the question whether it is solely NREM sleep, or the combination of NREM and REM sleep that produce the memory benefits following sleep. Based on a number of behavioral findings it has been suggested that SWS and REM sleep play complementary roles in memory processing (Stickgold et al., 2000; Gais et al., 2000; Ambrosini et al., 1995; Ambrosini & Giuditta, 2001; Ficca, Lombardo, Rossi, & Salzarulo, 2000). Also, it is important to consider the striking differences between REM sleep and SWS. REM sleep is characterized by a desynchronized, "saw-toothed" theta rhythm in the electroencephalogram (EEG), elevated acetylcholine (ACh) levels, suppressed monoaminergic tone, and heightened cognitive activity, while SWS is characterized by high amplitude, synchronous delta EEG, hippocampal sharp waves, and low ACh levels. While the underlying mnemonic mechanisms of the various sleep stages are not well understood, it is possible that REM sleep could modulate, disrupt, or further enhance NREM (especially SWS) processing of the memory trace.

The present study employs a daytime nap design that specifically isolates the effects of NREM sleep by allowing subjects to obtain only NREM without entering REM sleep. This design not only parses out the selective effects of NREM-related processing of different types of memory tasks, but it also allows for an assessment of the effects a comparatively brief period of sleep (a 1-hour daytime nap) on memory processing. Daytime naps have been shown to beneficially impact a number of performance variables (Takahashi, 2003), and it has recently been demonstrated that a daytime nap that contains

REM sleep is associated with performance improvements on a visual discrimination task (Mednick et al., 2003). However, to our knowledge, no studies have been conducted that demonstrate the importance of a NREM daytime nap for improved declarative, but not procedural, memory performance.

Given the evidence supporting a role for NREM sleep in the processing of declarative but not procedural memory, we expected that subjects that obtain NREM sleep (stages 2, 3, and 4), but not REM sleep, during a daytime nap would improve more on a measure of declarative memory (paired associates) than those remaining awake during a 6-hour training/retest interval. We also predicted that NREM sleep stage amounts (especially SWS) would correlate positively with improvement in paired associate recall. The degree of improvement on a procedural memory task (mirror tracing) was expected to be the same for nap and wake subjects.

Methods

Subjects

Subjects were 29 undergraduate students (13 males, 16 females) of diverse ethnic backgrounds from the City College of New York (ages 18-48, mean=23). All subjects reported being in good health and were not taking medications that might alter sleep architecture or ability to fall asleep. The 29 subjects (12 nap subjects (6 males, 6 females; ages 18-34, mean=20.1) and 17 wake subjects (7 males, 10 females; ages 18-48, mean=25.2) used for data analysis were selected from a larger initial sample of 33 subjects that completed the experiment. The 12 nap subjects obtained SWS but not REM sleep during the nap. Two subjects from the wake group were excluded from analysis

because of exceedingly low baseline scores on the paired associate task (> 2 SD from the group mean). One subject did not complete the digit span task, and another did not correctly perform the mirror tracing task, and were therefore excluded from these particular analyses.

Procedure

Three days before the study subjects signed a consent form, were asked to complete a demographic questionnaire, and also asked to complete a sleep log that recorded bedtime, wake time, and total sleep time prior to the day of the study. They were required to refrain from alcoholic and caffeinated drinks the night before and morning of the study. Subjects were also instructed to eat prior to arriving at the lab or to bring food with them to eat in the lab. Kitchen facilities in the laboratory were made available to subjects throughout the study. The day of the study, subjects arrived at 11:30am at the Laboratory of Cognitive Neuroscience and Sleep at the City College of New York. Upon arrival, subjects were familiarized with the sleep chambers and the nature of the study.

To create similar testing conditions prior to baseline learning, sleep and wake subjects had ten electrodes applied. At approximately 12:15pm subjects underwent the baseline learning phase of the experiment, which took place at two testing areas within the laboratory, one for the mirror tracing task, and one for digit span and paired associates. Digit span was always performed immediately before the paired associates task. These two tasks were counterbalanced with presentation of the mirror tracing task. The initial learning phase took approximately 30-40 minutes to complete.

At 1:00pm the sleep subjects were taken to a sound attenuated sleep chamber to attempt to take a nap (nap group). To address the possibility nap subjects might rehearse the information obtained during baseline learning during the period prior to sleep onset, wake subjects (wake group) were instructed to sit in a chair in another sleep chamber for a period of ten minutes to provide them an equivalent period for rehearsal. After this period of 10 minutes, electrodes were removed from the wake subjects. Wake subjects remained awake in the laboratory until the 6:00pm retest session and were allowed to engage in activities that included looking at magazines or watching a pre-selected movie. These passive activities were chosen specifically to minimize the acquisition of new declarative information that could interfere with memory performance at retest.

The sleep group was monitored with digital EEG acquisition software (Gamma System-Grass/Telefactor^{tm}) using a five-channel montage (two EEG (C3/A2, C4-A1), two electro-oculography (EOG), and chin electromyography (EMG)) to monitor sleep during the nap. Sleep stages were scored by a registered polysomnographic technician using the standard criteria (Rechtschaffen & Kales, 1968). The scoring technician was blind to subjects' performance on the memory tasks. Sleep subjects were permitted to attempt to sleep for a period of approximately one hour. If subjects obtained SWS during this time they were allowed to sleep until it appeared that the first SWS period was coming to an end. After the sleep period electrodes were removed and subjects remained in the laboratory until the retest session. The nap subjects engaged in the same activities as the wake subjects until the retest session. At 6:00pm all subjects were retested on the three tasks in the same order as during the baseline training phase.

Memory tasks

Paired Associates

Forty-eight semantically related word pairs (e.g., clock-hands) were selected from a larger pool of word pairs used by Plihal & Born (1997). Eight word pairs (four at the beginning and four at the end) were excluded from the response phase to account for primacy/recency effects. Word pairs were presented on a 15" VGA monitor for five seconds each with a 100 millisecond interstimulus interval (ISI). Immediately following presentation of the word pair list, subjects were shown in random order the first word of each of the 40 word pairs (minus the four at the beginning and four at the end) and asked to type in the word that completes the pair. After each response was entered the correct answer was displayed for two seconds. At retest subjects were shown the same 40 target words in a different random order, and were asked to type the word that completed the word pair.

In this study subjects performed just one learning trial, which differs from previous studies that had subjects repeat the response phase until they performed to criterion (usually 60% correct) (Plihal & Born, 1997, 1999a; Yaroush et al., 1971; Barrett & Ekstrand, 1972). In those studies some subjects completed the response phase only once if they reached criterion and others were required to complete the response phase a second time if the number correct was below criterion. Because it is not known whether one versus two or three exposures to the response phase at baseline affects performance at retest, independent of whether subjects sleep or remain awake, we had all subjects complete just one response phase. Our pilot work indicated this to be a more valid testing

approach even though it naturally comes at the expense of increased within groups variability on mean number of correctly recalled word pairs at baseline.

Performance was measured as the number of correctly completed word pairs, while improvement was measured as number of word pairs recalled at retest minus number recalled at baseline training. This raw score improvement was also calculated as a percentage improvement over the original baseline score.

Mirror Tracing

A five-pointed star was created with a ⅛" wide alley and points that extended 2¾" from the center (Fig. 3a). Subjects sat at a table on which the mirror tracing enclosure was anchored. Subjects placed their hands in an opening in the front of the enclosure that prevented them from directly viewing their hands. A mirror, positioned approximately two feet in front of the subject, allowed subjects to fully view their hands as they traced the star. The star was oriented with the 'start' point facing the subject with an arrow indicating the direction of movement around the star. To control for line thickness, all subjects used a 0.5mm ballpoint pen. Subjects were asked to work quickly, but to be as accurate as possible. Three mirror tracing trials were administered at baseline and at retest.

Performance was measured as average time per trial (in seconds) to complete the tracings, and as a measure of accuracy (amount traced outside the alley of each star measured in pixels). The measurement of mirror tracing accuracy employed here represents a novel approach to the issue that bears some similarity to a technique used in previous research (Plihal & Born, 1997). However, instead of measuring time spent

outside of the star's boundary, the number of pixels outside the boundary was calculated. This was done by scanning and saving each traced star image as a .jpg file (Fig. 3a). A mask was created that covered the alley of each star leaving exposed only the tracings that extended beyond the star's boundary (Fig. 3b). The number of black pixels was then counted using a pixel counter and averaged for the three baseline and retest star tracings. Reduction in time was measured as average time to complete the tracings at retest minus baseline, and improvement in accuracy was measured as number of pixels traced outside the star's boundary at baseline minus retest.

Digit span

The digit span test used in this study is based on the WAIS-III subtest using only the forward learning part of the test. It was used in this study as a general measure of attention at baseline and to acclimate subjects to the testing environment prior to performing the paired associates task. Numbers were presented serially on a 15" VGA monitor for one second each followed by a one second ISI. After the numbers were presented subjects wrote down on a response sheet the numbers in the order they were presented. The first test series consisted of three numbers with each subsequent series increasing by one number until the last series, which contained ten numbers. After responding to the last series of ten numbers, subjects then completed a second trial starting again with three numbers and progressing to ten numbers, but using different number sequences. Digit span always immediately preceded the paired associates task. The same task was administered at retest using different number series. Performance was measured as the number of correctly recalled number series at baseline and retest.

Results

A summary of sleep parameters for nap subjects are found in Table 1. The mean total sleep time (TST) was approximately 47 minutes, roughly 1/4 of the total sleep (approximately 190 minutes) obtained by subjects in the study by Plihal & Born (1997). Of the 47 minutes approximately 11% was spent in Stage 1, 41% in Stage 2, 21% in Stage 3, and 27% in Stage 4. Therefore, approximately 48% of the sleep period was spent in SWS (Stages 3 and 4), and approximately 90% of the nap was spent in stages 2, 3, and 4. Stage REM does not appear at all. Relevant pre-study sleep variables are represented in Table 2. Analysis of the three-day sleep log data revealed no differences between the nap and wake groups for number of hours awake prior to the baseline training session (t_{27}=.50, p=.62), total sleep time the night before the study (t_{27}=.26, p=.80), or the average total sleep time across the three nights prior to the day of the study (t_{27}=.13, p=.89).

Subjects in the nap group demonstrated greater improvement at retest than wake subjects, measured as the improvement in number of word pairs recalled at retest (8.75 ± .71 (mean ± SEM) versus 6.65 ± .71; sleep group x time interaction, $F_{1,27}$=4.16, p=.05; partial eta^2=.133) and percentage improvement over baseline ($F_{1,27}$=8.95, p=.006; partial eta^2=.25) (Fig. 1). Baseline recall of paired associates task for the nap and wake groups did not differ substantially (20.92 ± 1.78 versus 24.12 ± 1.14, p=.12). Pearson's product-moment correlations revealed positive, though non-significant, relationships between percentage of SWS in the nap and improvement on the paired associates task (% improvement - r=.37, p=.12; raw score improvement - r=.30, p=.17). The inverse was true when percentage improvement on the paired associates task was correlated with

percentage of stage 1 (r=-.26, p=.21) and stage 2 sleep (r=-.40, p=.10). Because of the difference in direction of the correlations for SWS and stages 1 and 2 with performance, the correlation between TST and percentage improvement was negligible (r=.03, p=.46).

Mirror tracing data revealed no differences between groups at baseline or retest. Nap and wake groups did not differ at baseline on average accuracy (number of pixels drawn outside the star boundary) (87.73 ± 13.23 versus 75.92 ± 14.28, p=.69) or average time to complete the three star tracings (119.06 ± 23.67 versus 113.71 ± 12.75, p=.83). There were no differences between groups on the two measures of improvement – improvement in accuracy (35.67 ± 8.89 versus 26.18 ± 6.67; sleep group x time interaction, $F_{1,26}$=.76, p=.39) or decrease in time to complete the star tracings from baseline to retest (-57.92 ± 17.36 versus -59.96 ± 10.33; sleep group x time interaction, $F_{1,26}$=.006, p=.94) (Fig. 2). Due to a significant positive skew in the distribution of our measure of tracing accuracy (amount drawn outside the star's boundary) for both subject groups, the data were square root transformed.

There was not a significant difference in the baseline digit span performance of nap and wake subjects, although mean performance for the wake group was slightly higher (5.16 ± .33 versus 4.35 ± .31, p=.09). Both groups demonstrated similar improvement from baseline to retest (.47 ± .19 versus .26 ± .25; sleep condition x time interaction, $F_{1,27}$=1.06, p=.67). Because digit span is a measure of immediate memory and known not to rely on the hippocampus for processing, performance was expected to be uncorrelated with performance on the paired associates. Pearson's product-moment correlations revealed that baseline digit span scores (r=-.109) and digit span improvement (r=-.042) were not correlated with improvement on the paired associates task.

Discussion

Using a research design that specifically isolates the effects of NREM sleep we show that improvement on a paired associates declarative memory task is greater for subjects that take an early afternoon nap than for subjects that remain awake during the baseline-retest interval. This difference in improvement is not observed for the procedural memory task, which lends further support to a growing body of evidence that NREM sleep plays a special role in the processing of declarative, but not procedural, memories. Recognizing that our findings are confined to a specific time period during the day, the results represent the first evidence that a brief daytime nap can significantly benefit declarative memory performance, the practical application of which should be appreciated. Traditionally, time devoted to daytime napping has been considered to be counterproductive in environments requiring mental acuity and substantial memory capacity. Clearly, this assumption may not be warranted given the performance benefits (Takahashi, 2003), procedural memory enhancement after naps containing NREM and REM sleep (Mednick et al., 2003), and now declarative memory improvement following a daytime nap containing only NREM sleep.

In addition to the practical ramifications of the current findings it is noteworthy that the statistical values representing the effect of NREM sleep on improvement in number of word pairs recalled and percentage improvement over baseline ($F_{1,27}$=4.16 and $F_{1,27}$=8.95) are similar in magnitude to the findings of Plihal & Born (1997) ($F_{1,18}$=4.79 and $F_{1,18}$=4.46) after a relatively brief period of sleep (47 minutes, compared to approximately 190 minutes in the study by Plihal & Born (1997). This similarity could be interpreted to mean that longer periods of sleep recorded in nocturnal studies are not

required for early processing of declarative memory. However, it is also possible that other factors, such as circadian influences, could also play a part in this enhanced processing. For example, cortisol levels, which are at their circadian nadir early in the nocturnal sleep period, have been shown to modulate recall of declarative information. In one study, infusion of cortisol during an early period of sleep, which elevated plasma cortisol to levels comparable to morning peak circadian levels, impaired recall of paired associates compared to subjects that received a placebo (Plihal & Born, 1999b). Sleep in the present study was recorded during a time when cortisol levels are at about their circadian midpoint (Weitzman, Schaumburg, & Fishbein, 1966), which may or may not have mediated the effect of sleep in the present study.

While there may exist subtle differences between diurnal and nocturnal sleep, it should be noted that the design for the present study was based on what is currently known about the general characteristics of NREM sleep-related physiology and its potential to facilitate declarative memory processing. Specifically, the role of NREM sleep for declarative memory processing is strongly tied to the necessary function of the hippocampus for declarative memory processing (Milner, Corkin, & Teuber, 1968; Squire, 1992). It is well known that elevated hippocampal ACh during wakefulness is important for encoding (Hasselmo & McGaughy, 2004), and that suppression of hippocampal ACh during encoding produces noticeable impairments in later retrieval (Rogers & Kesner, 2003). The inverse is true during SWS when low levels of ACh appear to be necessary for optimal consolidation of the memory trace. (Gais & Born, 2004) demonstrated this by artificially elevating ACh levels in humans during SWS by the intravenous administration of the ACh agonist physostigmine. They found that recall

was markedly paired impaired in this group compared to control subjects that received a placebo.

In addition to sleep dependent fluctuations in ACh, there are unique electrophysiological events that occur during SWS that may be intimately linked to information processing. During SWS, low ACh levels in the hippocampus produce a disinhibition of glutamatergic cell groups that generate periodic high amplitude sharp waves and high frequency ripples (~200Hz), which are a hypothesized mechanism for the integration of declarative information within neocortical targets by the hippocampus (Buzsaki, 1989, 1998). These sharp wave/ripple events have been shown to activate efferent projections to entorhinal cortex (Chrobak & Buzsaki, 1994, 1996) during SWS, and recent neurophysiological evidence suggests they are closely related to thalamically generated cortical spindles, which occur during stage 2 and SWS. In fact, it has been shown in rats that the occurrence of sharp waves/ripples is correlated with spindle activity in the medial prefrontal cortex (Siapas & Wilson, 1998), and somatosensory cortex (Sirota, Csicsvari, Buhl, & Buzsaki, 2003). While the temporal sequencing of these events has yet to be clearly defined, these findings nevertheless point to the potential importance of an interaction between thalamocortical spindles and SWS-associated hippocampal events (sharp waves/ripples) for sleep-dependent declarative memory processing.

While the physiological processes described above suggest a putative mechanism for sleep-dependent declarative memory processing, conclusive experimental evidence of the mnemonic function of individual sleep stages has yet to be documented. However, researchers have recently begun to manipulate cortical electrophysiology during SWS in

ways that impact memory processing. In a recent study it was shown that augmentation of the negative DC potential characteristic of the synchronous, high amplitude cortical electrical activity observed during SWS, by application of transcranial direct current stimulation (tDCS), produced a striking enhancement of declarative (paired associates) memory recall compared to subjects that did not receive tDCS (Marshall, Molle, Hallschmid, & Born, 2004). This finding elegantly demonstrates that sleep-related cortical electrophysiology may directly modulate post-sleep declarative memory performance.

Although the results of the present study are encouraging, we should bear in mind that they are preliminary and that there are a number of issues that should be addressed in the future to more clearly establish the role of NREM sleep for declarative memory processing. First, to strengthen the argument that sleep physiology does not merely permit memory consolidation but represents an active mechanism for memory formation, a strong correlation between amount of SWS and memory improvement should be established. To date, very few studies have reported correlations between sleep parameters and improvements in memory performance. In the present study correlations between SWS parameters and declarative memory performance were in the hypothesized direction, but fell short of statistical significance. It remains to be seen whether these non-significant correlations are a reflection of the limited amount of sleep obtained by nap subjects, the small subject sample size, or other influences specific to daytime sleep, such as yet unidentified circadian factors.

A second unresolved issue regards the lasting effects of sleep dependent memory enhancement. The present study used a baseline-retest interval of six hours, which

should have allowed for early consolidation processes to occur. However, retesting subjects after longer time intervals following a nap will inform us of the longer term effects of sleep following information acquisition. Mednick et al. (2003) showed that enhanced procedural memory performance on a visual discrimination task was maintained up to 72 hours after a nap. It remains to be seen if this finding translates to declarative types of memory.

III

Sleep Dependent Consolidation of Semantic and non-Semantic Declarative Information: The Importance of Adequate Encoding

Introduction

One of the most salient features of declarative memories (e.g., knowing the capitol of Vermont, or the name of the 42nd president) is that initial processing of the memory relies on the hippocampus. Over time, as the memory consolidates, the hippocampus becomes less necessary for memory recall as this function is assumed by neocortical circuits that are hypothesized to slowly "interleave" these new memories with previously learned information (McClelland, McNaughton, & O'Reilly, 1995; Frankland & Bontempi, 2005; Eichenbaum, 2000). Experimentally, it has been shown that a period of weeks or longer may be required for memory retrieval to become independent of hippocampal involvement (Zola-Morgan & Squire, 1990; Bontempi, Laurent-Demir, Destrade, & Jaffard, 1999; Squire & Alvarez, 1995). Because this period of memory consolidation necessarily includes cyclic bouts of sleep, there has been a broad effort to explain the possible role sleep plays in this protracted consolidation process (for reviews see Smith (2001) and Walker (2005)). Interestingly, early studies of the influence of sleep on memory processing largely viewed sleep as a passive state that merely prevented interference (or slowed the decay) of the memory trace (Jenkins & Dallenbach, 1924; Ekstrand, 1967). However, this conceptualization of sleep has been almost entirely supplanted by dynamic models of sleep-related memory consolidation as increasing

knowledge is gained about the underlying physiology of memory systems, and the richly complex biology of the sleeping brain.

One significant leap forward in our understanding of how sleep may represent a necessary state for optimal memory consolidation is based on the central hypothesis of a 'hippocampo-neocortical dialogue' (Buzsaki, 1989, 1998) that is expressed most robustly during slow wave sleep (SWS). The theory postulates that information encoded by the hippocampus during wakefulness is communicated to neocortical targets through the expression of sharp waves and high frequency (~200hz) ripple events (SPW-R), which are predominantly expressed through a process of disinhibition of hippocampal glutamatergic cell groups during SWS, when brain acetylcholine (ACh) levels are at their lowest (Hasselmo, 1999). During SWS these burst events propagate from the CA3-CA1 region out through deep layers (V-VI) of entorhinal cortex, suggesting a possible mechanism for the efferent communication of information from the hippocampus during sleep (Chrobak & Buzsaki, 1994). Complementing this finding, subsequent research demonstrated that principle hippocampal cells specifically involved in the encoding of waking spatial experiences participate in SPW-R events (Kudrimoti, Barnes, & McNaughton, 1999), and that SPW-R events are temporally correlated with cortical spindle activity during non-rapid eye movement (NREM) sleep (Siapas & Wilson, 1998; Sirota et al., 2003). These findings provide a preliminary framework for the general theory that sleep-specific brain physiology is specialized for memory consolidation processes (Buzsaki, 1989; Sejnowski & Destexhe, 2000).

Still needed, however, is a sustained effort to augment our growing understanding of the physiological basis of sleep-related memory processing with a more detailed

characterization of the memory changes that follow a period of sleep. While the effect of NREM sleep (and especially SWS) on declarative memory improvement is becoming better clarified (Plihal & Born, 1997, 1999a; Peigneux et al., 2004; Takashima et al., 2006; Tucker et al., 2006) there is still considerable debate about which types of declarative tasks, and which task stimulus characteristics, are enhanced by sleep (Vertes, 2004). For example, it has been suggested recently that NREM sleep may only improve memory for semantically related paired associates because the semantic relationships between the word pairs (e.g., clock-hands) are already well learned by subjects (i.e., strongly instantiated in neocortical networks), and that processing of these pairs could proceed independently of sleep-related hippocampal mechanisms (Stickgold, 2004, 2005). Viewed from this perspective, the basis for sleep-related memory facilitation is a general enhancement of cortical connectivity within pre-existing semantic cortical networks. While this is a reasonable assumption, it cannot be denied that acquisition of such semantically charged stimuli requires the hippocampus, as evidenced by patients with hippocampal lesions that exhibit a nearly complete inability to memorize this type of information (Squire, 1992; Milner et al., 1968). However, given the fact that almost every sleep/memory study has used stimuli that carry significant semantic weight, it cannot be ruled out that cortical structures may play an even more significant role than the hippocampus in the processing of the memory trace during sleep. Of course, if the hippocampus and neocortex are both integral to a process by which neocortical memory traces are strengthened by hippocampal outputs during sleep, then declarative tasks that lack pre-existing semantic associations should still benefit from NREM sleep. To test this idea, the present study examines performance on a semantically *unrelated* paired

associates task known to impose strong hippocampal resource demands during encoding. Imaging studies in humans have shown that when subjects are instructed to associate unrelated word triplets based on desirability hippocampal activation is significantly increased compared to when they are instructed to simply learn the words individually (Davachi & Wagner, 2002). Similarly, associating unrelated words increases hippocampal activation compared to deep encoding of individual words (Henke et al., 1999). If the hippocampus is involved in sleep-related memory processing (i.e., the integration of information within neocortical targets) then performance on a task known to be strongly dependent on the hippocampus for encoding should benefit more from a period of NREM sleep than has been shown in past studies using only semantically related paired associates (Plihal & Born, 1997; Tucker et al., 2006) that do not rely as strongly on the hippocampal apparatus for encoding.

While the use of semantically unrelated word pairs would increase hippocampal resource demands during encoding, thus supporting the idea that hippocampal processes active during sleep assist in the consolidation of the memory trace, the semantic richness of the individual words themselves would still strongly activate well-formed cortical networks during the learning phase. Because of the simultaneous hippocampal and cortical activation observed during encoding of such a task, one would expect the hippocampo-neocortical dialogue to be more robust during sleep following such a task, therefore leading to improved later performance. However, to better target the hippocampal nature of sleep-dependent memory processing, tasks that are largely devoid of semantic value should be employed. Doing so would clarify the extent to which hippocampal processes, acting without benefit of previously learned semantic

relationships, benefit performance following sleep. To date, only a few studies have examined the effect of sleep on non-semantic declarative memory tasks. Two studies have used the Rey-Osterrieth complex figure test (ROCFT) (a measure of visuospatial declarative memory) to assess the effect of sleep on memory in clinical populations. In epileptic patients it was shown that performance on the ROCFT correlates positively with spectral power in the low frequency EEG band (<1.25hz) (Bodizs et al., 2002), and in schizophrenic patients the amount of SWS correlates positively with ROCFT performance (Goder et al., 2004). In the present study subjects performed two non-semantic memory tasks: the ROCFT and a maze learning task adapted from the task used by Brenda Milner with a large number of hippocampal lesion patients, including the patient HM (Milner, 1965). Both of these tasks are void of semantically charged landmarks, objects, or verbal material that would have been previously learned and well-instantiated in cortical networks.

In addition to the use of declarative tasks that contain stimuli of differing semantic value, we considered the effect of contextual task properties of the paired associate task that may rely on hippocampal mechanisms for processing. It is well known that facts and experiences are not merely learned as isolated units, but within a larger context that provides a rich spatiotemporal framework for those experiences (Rudy & Sutherland, 1989; Eichenbaum & Cohen, 2001). The importance of the hippocampus for processing of contextual elements has been demonstrated numerous times with contextual conditioning paradigms in which the entire environment is changed (Rudy, Barrientos, & O'Reilly, 2002; Penick & Solomon, 1991; Phillips & LeDoux, 1992; Mumby et al., 2002; Kim & Fanselow, 1992). However, there has only been one study to our knowledge that

has examined the benefits of sleep for consolidation of contextual information (Drosopoulos et al., 2005). This study showed that when a simple contextual cue (font) was altered at retest, sleep facilitated accurate recognition of more same-context words compared to wake subjects that remembered a similar number of same and different context words. This finding suggests that sleep-related hippocampal processes consolidate aspects of the context that were encoded during baseline learning. The present study applies a similar context shift to a recall task (the unrelated paired associates task) by manipulating font, font color, and background color to create a more salient learning context.

Finally, an important issue that has received little attention in the literature thus far is how differences in the encoding of paired associates modulate the effect of sleep on memory. Almost all studies using paired associates have used a 'study-test' paradigm, whereby subjects learn word pairs followed by a number of test sessions until subjects reach a specified performance criterion (e.g., 60% correct or one perfect recall trial) (Yaroush et al., 1971; Gais & Born, 2004; Plihal & Born, 1997). The number of words recalled is used as a baseline measure of memory that can be compared to a later test session following a period of wake or sleep. While it has been demonstrated a number of times using this method that sleep facilitates recall of word pairs, the effects on later performance of a variable number of test sessions has not been adequately addressed. In a previous nap study we attempted to control for this effect by having subjects perform only one test session during the learning phase (Tucker et al., 2006). However, it may be that the effect of sleep is weakened by testing subjects on the same set of words during baseline encoding and at retest (i.e., subjects may have overlearned the word pairs during

the learning session). To add to our understanding of the nature of information encoding and its potential to modulate the effects of sleep on memory, we tested subjects on only 1/3 of the word pairs during baseline learning, while the other 2/3 were studied only. The tested word pairs served as a covariate for analysis of the studied word pairs.

To further characterize the mnemonic benefits of hippocampo-neocortical processes active during NREM sleep, we examined the above issues using a daytime nap paradigm, in which subjects obtained NREM including SWS, but did not enter REM sleep. First, we expected that recall of both studied and tested unrelated word pairs would benefit more from a brief bout of NREM sleep compared to an equivalent period of wakefulness, and that this effect of sleep would also be evident in two non-semantic, hippocampus-dependent declarative memory tasks (ROCFT and maze learning). Second, to add to the findings of (Drosopoulos et al., 2005) three contextual cues (letter font, letter color, and background color of the unrelated paired associates) were manipulated in the unrelated paired associates task. If contextual cues are preferentially consolidated during sleep nap subjects should recall proportionally more word pairs presented in the same context vs. changed context at retest compared to wake subjects.

Methods

Subjects

From an original sample of 39 undergraduate students, 33 subjects (11 males, 22 females, mean age=23.3) of diverse ethnic composition participated in the study. Six of the original 39 subjects were excluded from analysis because they either did not obtain SWS (n=3), they entered REM sleep (n=2), or did not sleep during the nap period (n=1).

All subjects were medication free, and abstained from caffeine and alcohol 24 hours prior to participation. A sleep log was obtained from all subjects indicating bedtime, wake time, and total sleep time for the three nights prior to the study. All subjects were paid for their participation.

Procedure

Subjects arrived at the sleep laboratory at 11:30am. Between 11:30am and 12:00pm subjects were shown the laboratory sleep chambers, signed a consent form, and completed a demographic information form. At 12:00pm nine electrodes were applied to record sleep, including central electroencephalography (EEG; C3-A2 and C4-A1), electro-oculography (EOG), and chin electromyography (EMG) leads. To create similar experimental conditions, all subjects, including wake subjects, had the same nine electrodes applied, and subjects were not informed of group assignment (nap or wake) until after the baseline learning session. Baseline learning on the three declarative memory tasks took place from 12:15pm to 12:45pm. The order of task presentation was fully counterbalanced across subjects. Approximately 15 minutes after the learning session (1:00pm), nap subjects entered individual sleep chambers to take a nap. At the start of the nap opportunity, wake subjects entered another sleep chamber and sat quietly for a period of 10-12 minutes, a time period comparable to that experienced by nap subjects prior to sleep onset. This condition was imposed primarily to address the issue of possible rehearsal of information that might occur with nap subjects prior to sleep onset. After this 10-12 minute period, wake subjects were taken to a separate room to watch a television program (e.g., an episode of Seinfeld) until nap subjects joined them at

approximately 2:00pm. Nap subjects attempted to sleep for a period of approximately one hour. If subjects entered SWS they were allowed to sleep until it appeared the SWS period was ending or it appeared they were entering REM sleep. All subjects were awakened from stage 1 or 2 sleep, or after an arousal from sleep. After the sleep period nap subjects joined the wake subjects to watch a movie until the retest session at 4:00pm. At 4:00pm all subjects were retested on the same three tasks presented in the same order as during baseline learning.

Memory tasks

Semantically unrelated paired associates

Sixty word pairs were created from common objects (e.g., 'Computer' and 'Nose'), which were randomly paired to eliminate any semantic relatedness between the pairs. Subjects were instructed to use a mnemonic strategy to help them learn the pairs. For the example word pair ('Alligator-Cigar') subjects were instructed to visualize the two words interacting with each other, such that they might imagine an alligator smoking a cigar. Each word pair was presented for two seconds with a 100ms interstimulus interval (ISI). All word pairs were presented in Times New Roman font (font size=54). Three contextual features were built in to each word pair slide, so that slides alternated between 1) blue, non-italicized text against a gray background, and 2) yellow, italicized text against a black background. After presentation of all word pairs, subjects completed a test phase during which they were presented the stimulus (first) word of 20 of the word pairs (randomly selected from the 60 presented pairs), and were asked to type the target word that went with it into a text box. After typing in their responses to the 20 tested

word pairs subjects viewed all 60 word pairs once more, this time presented each for one second, with an ISI of 100ms. At retest subjects were shown, in random order, the stimulus words for all 60 word pairs, and were asked to recall as many of the target words as possible.

Maze learning task

The maze task is a computerized version of the "bolt head maze" used by Milner with the patient HM and a large number of hippocampal lesion patients (Milner 1965). Our maze is an 11x11 array of squares, each representing either a correct step in the path of the maze, or a wall. Subjects start at the 'start' square in the lower left hand corner and move left-right or up-down (but not diagonally) clicking each neighboring square with a mouse. If a subject is on the correct path, each square lights up green, and if the subject hits a 'wall' the square lights up orange. With each forward mouse click the preceding square returns to its original gray color. The first time through the maze subjects progress blindly from start to finish, but with each subsequent trial, subjects commit more of the path to memory. During baseline learning subjects completed five maze trials, recording on a response sheet the trial time and number of errors at the end of each trial. At retest subjects completed eight maze trials.

Complex figure test

The Rey-Osterrieth complex figure test (ROCFT) is a standard neuropsychological test used primarily as a clinical assessment tool to screen for brain injury that also measures visuospatial integration capacity and short-term visual memory

(Osterrieth 1944). In this study subjects were presented the complex figure (see Figure 4) and were given five minutes to copy the entire figure onto a blank sheet of paper. If subjects finished copying the figure before the allotted time they were instructed to go over their work for the remainder of the time. At retest, subjects were again given five minutes to redraw the figure from memory. Administration of the ROCFT differed from traditional methods in that recall was assessed approximately four hours after the baseline session (instead of the usual 20 minute interval), and all subjects were informed that that they would be retested on the figure later in the day. Scoring of the complex figure was based on a modified version of the Boston Qualitative Scoring System (BQSS) (Stern et al. 1999), such that the six configural elements, nine clusters, and six details were given a 0, ½, or 1 point score. Zero was scored if less that half of the part was represented, or both orientation and position criteria were violated. A score of .5 was given if greater than 50% but less than 100% of the part was represented or if only orientation or position was violated. One point was given if 100% of the part was represented and orientation and position criteria were met. A total of 21 points could be obtained on this task.

Results

Sleep Data

Sleep data are presented in Table 3. Subjects were included in the final sample if they obtained SWS (but not REM sleep) during the nap. Sleep log data revealed no differences for amount of time awake prior to the study ($p=.47$), total sleep time the night before the study ($p=.28$), and average total sleep time for the three nights prior to the study, ($p=.17$) (Table 4).

Unrelated Paired Associates

Performance on the 20 tested pairs during the learning session was similar for both groups (nap group: 7.4 ± 0.9, wake group: 9.4 ± 1.3, t_{31}=1.31, p=.2). This baseline performance score was used as a comparison for tested word pairs at retest and as a covariate for analysis of the 40 studied word pairs. There was no difference in recall at retest between nap and wake groups for the 40 studied word pairs, (nap group: 8.6 ± 1.1, wake group: 9.2 ± 1.8 (mean ± SEM), one-way ANCOVA, $F_{1,30}$=1.60, p>.2) (Figure 5). However, for the nap group performance on the tested word pairs significantly improved from baseline learning to retest (+1.6 ± 0.7, t_{15}=2.23, p=.04), and this improvement was significantly greater than performance in the wake group, which demonstrated mildly impaired recall at retest (-0.3 ± 0.4; sleep group by time interaction, $F_{1,31}$=5.26, p=.03) (Figure 5). Overall, the percentage of tested word pairs correctly recalled at retest (42.4 ± 3.9%) was greater than for studied word pairs (22.3 ± 2.6%), t_{32}=8.23, p<.001). When subjects were divided into equal groups of high and low performers based on a median split of performance of the 20 tested word pairs during the learning session, sleep-related improvement in recall was greater for high performers (sleep group by time interaction, $F_{1,15}$=5.66, p=.03), but the nap and wake subjects in the low performing group improved similarly (sleep group by time interaction, p>.4). Regardless of whether subjects were categorized as high or low performers, the nap and wake groups recalled a similar number of untested word pairs at retest (one-way ANCOVAs, p>.2).

Context

Context analysis of the 20 tested word pairs revealed that nap and wake subjects recalled the same proportion of same context versus different context pairs (nap group: 50.4 ± 3.9%, wake group: 47.8 ± 5.4% same context word pairs, t_{31}=.38, p=.70). However, for the 40 untested pairs wake subjects recalled a smaller proportion of same context word pairs than nap subjects (50.5 ± 3.5% versus 36.6 ± 4.4%, t_{31}=2.50, p=.02).

Maze Learning

The nap and wake groups performed similarly during baseline learning for number of errors committed per trial (nap group: 39.2 ± 5.3, wake group: 31.3 ± 4.3, t_{31}=1.17, p>.2) and average time to complete each trial (nap group: 103.0 ± 9.6, wake group: 90.9 ± 8.3, t_{31}=.96, p>.3). Both groups improved significantly from baseline to retest for reduction of errors and for average time to complete each trial (all paired samples t-tests for nap and wake groups, p<.001)). Overall, the nap group did not improve more than wake group for reduction of errors (sleep group by time interaction $F_{1,31}$=1.13, p=.30; Figure 6) or time to complete each maze trial (sleep group by time interaction $F_{1,31}$=1.06, p=.31) (Figure 7). However, when subjects were divided into high and low performers based on a median splits of number of errors and time per trial during learning there was a significant sleep group by time interaction in the high performing group for reduction in errors ($F_{1,14}$=10.81, p=.005; Figure 6) and time per trial ($F_{1,14}$=6.03, p<.03) at retest (Figure 7). For the low performers, all sleep group by time interactions were not significant, p>.5 (Figures 6 and 7).

Complex Figure

During baseline learning all subjects copied the complex figure within the 5-minute time limit except three, who were excluded from this analysis. Of these three, two did not complete the figure within the 5-minute time interval, and one inadvertently did not complete the figure at retest. Overall, nap subjects recalled more of the complex figure at retest than wake subjects, but the difference did not reach significance (nap group: 14.8 ± 1.11, wake group: 13.7 ± .82; t_{28}=.75, p>.4; Figure 8). However, when the sample was divided into high and low performers based on a median split for complex figure recall at retest, nap subjects recalled significantly more of the complex figure than wake subjects (nap: 17.9 ± .40, wake: 16.6 ± .47; t_{13}=2.14, p=.05; Figure 8). Recall for the nap and wake groups was similar for low performers, p>.9; Figure 8).

Discussion

Several studies have shown that recall of semantically related word pairs improves more after a period of sleep compared to an equivalent period of wakefulness (Plihal & Born, 1997; Gais & Born, 2004; Tucker et al., 2006). The present study extends this general finding by examining the effect of a daytime nap on recall of paired associates that lack an intrinsic semantic tie. Surprisingly, performance on the word pairs that were only studied during baseline learning did not differ between nap and wake subjects. However, for the tested word pairs there was a marked improvement in recall for the nap group only, while recall in the wake group was slightly impaired at retest. We proffer the hypothesis that a test session immediately following the learning of word pairs does not produce overlearning during the learning session, but serves to more effectively

prime hippocampal and neocortical circuits for sleep-specific processing in a manner not well-suited to processing by the brain during wakefulness. Interestingly, it has been shown a number of times that a testing phase immediately following information encoding, as compared to a study phase only, greatly facilitates later recall (Roediger & Karpicke, 2006; Carrier & Pashler, 1992). The findings of the present study suggest that it may be the interposition of sleep between baseline learning and retest that explains this effect, as subjects in these studies experienced an intervening period of sleep before being retested.

While the sleep-related neurophysiological mechanisms of this improvement gain are not well understood, based on the nature of the hippocampo-neocortical dialog occurring during sleep, one implication of the study-test method may be that cortical networks activated as a function of immediate recall would be primed for later SPW-R activation, as is persuasively suggested in theoretical accounts of sleep-dependent memory processing (Buzsaki, 1998; Sejnowski & Destexhe, 2000). Indeed, a number of imaging studies reveal that specialized cortical regions (especially inferior prefrontal cortex) activated during the encoding of word stimuli (Kapur et al., 1994; Kahn et al., 2005) are reactivated during immediate retrieval (Dupont, Samson, Le Bihan, & Baulac, 2002; Nyberg, Habib, McIntosh, & Tulving, 2000; Buckner & Koutstaal, 1998), possibly strengthening the initially activated cortical circuits. The upshot of this proposed mechanism is that the effect of sleep should be more pronounced if, indeed, this testing phase more strongly primes neocortical networks such that hippocampal outputs more robustly target these specific neocortical targets. Of course, this notion remains speculative, and awaits empirical confirmation. Nevertheless, it is concordant with the

idea that sleep-related hippocampo-cortical communication is necessary to optimally strengthen the memory trace, a process expressed primarily during SWS (Buzsaki, 1998).

The findings of the present study also highlight the differences between the effects of sleep on related vs. unrelated word pairs. While most studies using semantically related word pairs have shown that while sleep subjects improve more than wake subjects at retest, performance in both groups improves over time (Plihal & Born, 1997; Gais & Born, 2004; Tucker et al., 2006). However, a recent study using the study-test method revealed that a full night of sleep facilitates recall of unrelated word pairs while recall is impaired following an equivalent (12-hour) period of wakefulness (Payne et al., 2006). This result parallels the results of the present study demonstrating that performance after encoding of unrelated word pairs only improves for subjects that obtain NREM sleep prior to retest. One possible explanation for the differences in the pattern of performance between sleep and wake subjects observed for related versus unrelated word pairs is that the increased hippocampal demand imposed by forming associations between unrelated words (Davachi & Wagner, 2002; Henke et al., 1999) better primes the brain for sleep-related memory processing, but because hippocampal transmission of information is muted during wakefulness the end result is impaired performance. Overall, the results of the present study fit well with the idea that when hippocampal demands are increased, and neocortical networks are activated during declarative information encoding, as with processing of word pairs, a hippocampo-neocortical dialogue is established that operates primarily during NREM sleep to optimally consolidate the memory trace (Buzsaki, 1989; Sejnowski & Destexhe, 2000), therefore leading to enhanced performance. However, while the effects of NREM sleep on semantically

charged stimuli (i.e., words) are becoming better established, we were also interested in evaluating the effect of sleep on declarative tasks that are virtually void of semantic value. For this reason we also used tasks that lack semantically charged stimuli (e.g., common words), objects, or associations that may have been previously learned by subjects.

Interestingly, the findings for the non-semantic declarative memory tasks (ROCFT and maze learning) did not reveal differences between the nap and wake groups overall, which was unexpected given the declarative (hippocampus-dependent) nature of the tasks. This finding suggests that a necessary condition for optimal sleep-related memory processing be that the encoded stimuli have well-learned semantic cortical representations. With this said, however, it is of special interest that, for each of these tasks, strength of encoding is a key modulator of the effect of sleep, such that sleep preferentially facilitates performance only for subjects that performed well on the tasks during baseline learning (high performers). That the encoding effect generalizes to both non-semantic declarative memory tasks is clearly important, but what remains to be discovered is why sleep is an effective mnemonic state only when information encoding is robust. Several hypotheses seem plausible: It may be that general intelligence, or some aspect of intellectual ability, is a factor that enables some subjects to more strongly encode information, which leads to an enhanced effect of sleep on consolidation. Alternatively, it may be that some subjects are more motivated to perform the tasks, either due to experimental demand characteristics, or because some subjects found the tasks more intrinsically rewarding. Future experiments should attempt to better characterize the basis for these encoding differences and the mechanisms by which sleep

facilitates the processing of strongly encoded information, while having little effect on weakly encoded material. Certainly, an intriguing and possible explanation for these results is that sleep serves the adaptive function of preferentially facilitating memory consolidation only for memory tasks that subjects are motivated to learn, and therefore become strongly encoded, while having little, if any, effect on information that is weakly encoded.

While there is a strong encoding effect associated with the sleep-related performance gains on the two non-semantic memory tasks, it should be appreciated that it was not necessary for subjects to be in the high performers on the paired associates task during baseline learning to observe the effect of sleep, even though a similar pattern emerged when subjects were divided into high and low performers. If memory processing is dependent on hippocampal and neocortical networks working in harmony during sleep (Buzsaki, 1998, 1989), it is reasonable to hypothesize that a task (e.g., paired associates) that activates hippocampal circuits as well as strong pre-existing cortical networks during a study-test encoding procedure could preferentially enhance hippocampo-neocortical communication during sleep. Tasks that lack strong semantic, (previously learned) cortical representations may only benefit weakly because hippocampal processes largely act in isolation during sleep. This may have been true with the ROCFT and maze learning tasks, which comprise stimuli that would likely not have been previously learned and well instantiated in cortical networks. However, one way to counteract this deficit in semantic value would have been for subjects to better encode the tasks during baseline training, which is, in fact, what the results of the present study reveal. While this is a suggestion that awaits further empirical validation, the results, overall, suggest that use of

tasks that vary in semantic quality may enlighten us about how hippocampus and neocortex work together in a task-specific manner to modulate sleep-dependent memory consolidation.

In addition to task stimulus properties we were also interested in the effect of sleep on consolidation of contextual information in the paired associates task. The findings for tested word pairs revealed that the nap and wake group recalled an equal number of same and changed context word pairs (~50%), which strongly suggests that the contextual cues were not salient enough to be preferentially consolidated by either group. This may have been because two of the cues (text color and shape) were embedded in the words themselves, and the only non-textual cue (background color) may have represented too subtle a change (from gray to black). Interestingly, for the studied word pairs, subjects in the wake group recalled proportionally fewer same context word pairs than the nap subjects, while nap subjects again recalled equal amounts of same and changed context word pairs. The reason for this is difficult to interpret, because instead of interfering with recall, the changed context facilitated recall (~65% of recalled word pairs were from the changed context). It could be that, for yet unspecified reasons, only weakly encoded contexts have an interfering effect on later recall. (It should be noted that the duration of initial encoding in the Drosopoulos et al (2005) study was approximately three times as long with seven times the number of stimuli used in the present study.) Future research, therefore, should attempt to balance the intensity of the encoding process with a richer array of contextual cues (e.g., multi-color and multi-pattern backgrounds) that create a more salient contextual backdrop during stimulus encoding.

Overall, the findings demonstrate that a brief bout of NREM sleep obtained during a daytime nap facilitates memory for unrelated paired associates, and that this improvement is related to how the word pairs were encoded. Results for the non-semantic declarative tasks, on the other hand, show that sleep-dependent memory facilitation depends on how strongly subjects initially encode the information. Naturally, it remains to be seen whether performance on non-semantic declarative memory tasks, independent of how strongly the tasks were encoded, benefits more from longer periods of sleep that contain both NREM and REM sleep (e.g., after a full night of sleep). To more fully understand the role of sleep for memory processing, it is important that scientists not only manipulate sleep duration, but that they also use a broader range of declarative tasks that include those of a non-semantic nature. As we show here, encoding procedures should also be seriously considered as modulators of the effect of sleep on memory.

IV

The Effect of Sleep Duration and Subject Intelligence on Declarative and Motor Memory Performance: How much is enough?

Introduction

The contributions of specific stages of sleep to the consolidation of different classes of memory are becoming better delineated. Research findings clearly implicate a role for REM sleep in the processing of procedural/perceptual memory (Karni et al., 1994; Stickgold et al., 2000; Smith & Lapp, 1991), while more recent findings shed light on the mnemonic significance of non-REM (NREM) sleep. Slow wave sleep (SWS) has been shown several times to be associated with enhanced recall of declarative information (Plihal & Born, 1997, 1999a; Gais & Born, 2004; Takashima et al., 2006; Peigneux et al., 2004), while stage 2 sleep appears to be important for the processing of motor skill tasks, such as number sequence learning (Walker et al., 2002; Walker et al., 2003) and pursuit rotor performance (Smith & MacNeill, 1994; Smith & Fazekas, 1997). The specificity of these findings is striking for its consistency across studies, and has led to a broad effort from multiple disciplines to better describe the physiological mechanisms responsible for these stage-specific processes. However, another approach to the issue that has not received much attention is an examination of the memory benefits provided not simply by qualitative aspects of sleep, but by sleep quantity. If one of the functions of sleep is to consolidate information acquired during wakefulness, then the assessment of varying amount of sleep on memory performance seems integral to a clearer understanding of this phenomenon. Thus far, several studies have described the

effect of a full night of sleep on changes in memory recall. For example, studies have shown that performance on declarative memory tasks after a night of sleep can either be very similar to presleep performance (Schabus et al., 2004; Gais, Molle, Helms, & Born, 2002) or can improve following a night of sleep (Payne et al., 2006), depending on how the task is encoded. Motor skill performance has also been shown to improve dramatically after a night of sleep compared to equivalent periods of wakefulness, and has been shown to correlate with the amount of stage 2 sleep during the night (Walker et al., 2003). However, specific manipulations of sleep duration are lacking in the literature, making it difficult to draw conclusions about the amount of sleep that is required to obtain optimal improvement in performance. Interestingly, daytime nap studies have shown that performance on a visual discrimination task (a test of perceptual memory) improves dramatically after a nap containing REM sleep (Mednick et al., 2003), while paired associate performance improvement has been shown to benefit following a nap containing only NREM sleep (Tucker et al., 2006). Might these findings imply that while sleep is important for memory formation, only small amounts are required to observe noticeable benefits? If so, then what would be the adaptive value of larger quantities of sleep for memory processing? To assess the effect of differing quantities of sleep on memory, the present study examines performance on a standard declarative memory task (semantically related paired associates) and a motor learning task (number sequence learning) after a half night of sleep (approximately 3.5 hours) and after a full night of sleep (approximately 7.5 hours). This manipulation of sleep serves two purposes. First, by examining these durations we can better understand the extent to which the memory trace continues to be processed over lengthier periods of sleep, or whether consolidation

processes plateau as might be suggested by the nap study findings. The other major purpose for such a manipulation would be to better clarify the effect of sleep duration on different memory systems. A number of studies have already found that sleep early in the night, which is dominated by SWS promotes the recall of declarative material (Plihal & Born, 1997; Gais & Born, 2004), while sleep obtained late in the sleep period, which is saturated by REM sleep and Stage 2 sleep does not appear to benefit recall of this type of information. Conversely, motor skill learning appears to benefit from Stage 2 sleep, the bulk of which is obtained late in the sleep period (Smith & MacNeill, 1994; Smith, 2001; Walker et al., 2002). Based on these findings, we put forth the tentative hypothesis that declarative memory performance will improve after 3.5 hours of sleep, while a full night of sleep should not further augment the recall observed after 3.5 hours of sleep. Motor skill performance, on the other hand, should not benefit substantially after the first 3.5 hours of sleep, but should be greatly enhanced after a full night of sleep, by virtue of increased amounts of Stage 2 sleep during the latter half of the night.

In addition to the effect of sleep duration on performance across different memory domains, this study also set out to assess the relationship between a subject's ability to encode information and changes in memory following sleep. A recent study looking at the effect of a daytime nap on semantic and non-semantic declarative memory tasks showed that the effect of sleep on memory is modulated by strength of information encoding (Tucker & Fishbein, submitted for publication). The study showed that sleep enhanced memory for a semantic declarative memory task (unrelated paired associates) compared to subjects that remained awake during the learning-retest interval, but memory was only improved on the non-semantic memory tasks (the Rey-Osterrieth complex

figure test and a maze learning task) if subjects strongly encoded the information. This encoding effect also applied to paired associates performance, but it was not a necessary condition to observe the sleep effect. From these results we hypothesized that specific subject variables might be responsible for encoding facility and, therefore, improved memory performance following sleep.

One subject variable that may be related to encoding proficiency and, therefore, amplified sleep-related memory processing is intelligence, which has already been shown to be related to sleep-related physiology. One pilot study showed a strong correlation between IQ, as measured by the Multidimensional Aptitude Battery-II (MAB-II; Jackson, 1998), and number of Stage 2 sleep spindles across the night (Nader & Smith, 2001). Notably, they found the highest correlations for Performance IQ ($r=.71$, $p=.02$) and Full Scale IQ ($r=.76$, $p=.01$), with a smaller correlation for Verbal IQ ($r=.56$, $p=.09$). The correlations were even more pronounced for sleep spindles occurring during the last two thirds of the night, which accounted for 85% of the variation in Performance IQ scores. This finding is complemented by a similar study showing a strong correlation ($r=.77$, $p=.0001$) between spindle density (especially in frontal regions) and scores on the Ravens Progressive Matrices Test (RPMT) (Bodizs et al., 2005).

While these findings suggest that general intelligence is related to “native” (invariant) sleep-specific physiology, there are only a few studies that have attempted to link intelligence with sleep-related memory gains. One study found that intelligence, as assessed by the RPMT, was not related to sleep-related memory improvement (Schabus et al., 2006). However, when subjects were placed into high and low memory groups based on scores on the Wechsler Memory Scale (WMS), they found that high memory

group performance benefited more from sleep, further suggesting that subjects that have a general encoding advantage experience greater sleep-related performance gains. Unfortunately, while these researchers had subjects perform a declarative (paired associates) and procedural memory task (mirror tracing) they did not present the specific results for each of the tasks. In another study subjects with higher IQ scores performed better than subjects with lower IQs on two procedural memory tasks during encoding and exhibited higher post-acquisition rapid eye movement densities (Smith, Nixon, & Nader, 2004). However, this study did not present findings associating intelligence with performance changes following sleep.

The present study sets out to add to the findings of these two studies by examining the influence of intelligence on encoding and sleep-related changes in memory performance over time using the MAB-II, a 10-subscale multiple-choice test that is highly correlated with the subscales of the Wechsler Adult Intelligence Scale III-R (WAIS III-R), and from which Performance, Verbal, and Full Scale IQ values can be obtained. Use of the MAB-II will allow for a more detailed analysis of the relationship between these variables that may not have been fully appreciated in previous studies.

Methods

Subjects

Twenty-four undergraduate students (12 female, 12 male; mean age=20.9) of diverse ethnic composition participated in the study. Eleven subjects were assigned to the ½ night sleep group and 13 to the full night sleep groups. All subjects were in good health, medication free, and abstained from alcohol and caffeine the day of the testing

session and the day of the overnight portion of the study. Subjects completed a sleep log to document their average bedtime, wake time, and number of hours of sleep for the three nights prior to the overnight study. All subjects reported a habitual bedtime before 12:00am. Subjects were paid and received course credit for their participation.

Procedure

Subjects were scheduled for a 1.5 hour test session prior to the overnight study to acclimate them to the laboratory environment and to take the Multidimensional Aptitude Battery-II (MAB-II) (Jackson, 1998), a 10-subtest multiple choice test of general intelligence. Several of the subtests of the MAB-II are similar to those of the WAIS III-R, and the Performance, Verbal, and Full Scale IQ values strongly correlate with the WAIS III-R scales (Jackson, 2003). The MAB-II is a timed test in which subjects are allowed seven minutes to complete each subtest.

Upon arrival at the sleep lab subjects were shown the lab facilities, signed consent forms, and were informed about the requirements of the study. They were then shown to a well-lit, sound attenuated room (one of the sleep chambers in the sleep laboratory) where they sat at a desk to await further instructions over an intercom. On the desk were the test booklet, two pencils, and the test answer sheets. A researcher that sat outside the room gave instructions for each subtest over the intercom, and encouraged subjects to ask questions over a microphone situated on the desk in each room. All subjects were video monitored during the test session to ensure compliance with test instructions. The researcher signaled when to start and stop each test. After subjects completed the test,

they were informed that a researcher would contact them by phone or email to set up an appointment for the overnight study.

On the night of the overnight study subjects arrived at the sleep lab at 9:30pm. Subjects completed a demographic questionnaire and the Epworth Sleepiness Scale (ESS) (Johns, 1991). After completing the forms, nine electrodes were applied to monitor sleep (two central EEG (C3-A2, C4-A1), two EOG, and one chin EMG channels). Sleep data were acquired using Grass-Telefactor Gamma software. After electrode application, subjects relaxed and watched a movie before the learning session. At 10:45pm subjects sat at a computer in a sound-attenuated room. The Stanford Sleepiness Scale (SSS) was administered to assess level of sleepiness at that moment. Subjects were then administered the digit span task as a measure of general concentration, and to mentally prepare them for the subsequent learning tasks. Following the digit span task, the paired associates task and motor sequence learning were administered in a balanced fashion across subjects. Subjects were individually instructed on all tasks, and were left to perform each of the tasks after indicating they understood the instructions. After performing each task, subjects informed the researcher, who instructed them on the next task. After the learning session, which took approximately 40 minutes to complete, subjects remained awake for another 15-20 minutes, during which time they were informed about when they would be awakened, either half way into the sleep period or after a full night of sleep. Subjects in the half night condition were told that they would be able to sleep several hours before being awakened.

Prior to retest, subjects were awakened either directly following an arousal from sleep, or from Stages 1 or 2 sleep. Subjects were never awakened from SWS or REM

sleep. After awakening, electrodes were removed and subjects were instructed to wash their faces, and to remove any residual electrode paste. This instruction was given as a means of activating subjects for subsequent testing. The retest session never occurred until at least 25 minutes had passed after awakening, to bring subjects to a fully waking biochemical brain state, and to eliminate performance decrements due to sleep inertia.

At retest, each subject completed the SSS and then completed each memory task in the same order as during baseline learning. After the retest session subjects completed the Student Opinion Scale (SOS; (Wolf & Smith, 1995)), which surveys subjects' interest in their performance and general motivation regarding the study. After completion of the SOS subjects were paid for their participation. Subjects in the half-night sleep group were then allowed to sleep out the rest of the night in the lab, while full-night sleep subjects were allowed to leave the lab.

Memory Tasks

Paired Associates

Forty-eight semantically related word pairs (e.g., clock-hands) were selected from a larger pool of word pairs used by Gais & Born (2004), and have been used in a previous study from our lab (Tucker, et al., 2006). Eight word pairs (four at the beginning and four at the end) were excluded from the response phase to account for primacy/recency effects. Word pairs in this task are presented on a 15" VGA monitor for five seconds each with a 100 millisecond interstimulus interval (ISI). Immediately following presentation of the word pair list, subjects are shown in random order the first word of each of the 40 word pairs (minus the four at the beginning and four at the end) and are

asked to type in the word that completes the pair. After each response is entered the correct answer is displayed for two seconds. At retest subjects are shown the same 40 target words in a different random order, and are asked to type the word that completes the word pair. Performance is measured as the number of correctly completed word pairs, while improvement is measured as number of word pairs recalled at retest minus number recalled at baseline training. This improvement score is also calculated as a percentage improvement over the original baseline score (# improved/# correct during baseline learning).

Motor Sequence Learning

The motor sequence learning task used in the present study was modeled on the task used by Walker (Walker et al., 2002; Walker et al., 2003). A 5-digit series of numbers (4-1-3-2-4) is displayed in black font 20 times in a window in the center of a 15" VGA monitor. As each correct number of the sequence is pressed the font changes to blue. After all 20 number sequences are typed the time and accuracy (# of incorrect keystrokes per 20-sequence trial) for the trial is displayed. Subjects are instructed to enter these values on a response form. The first trial is a practice trial to make certain each subject understands how to perform the task. Following the practice trial, subjects perform 11 trials on their own, and record the time and accuracy for each trial. To ensure optimal performance subjects are instructed to type as fast as they can, but to also strive for accuracy. At retest, subjects perform three trials of the task.

Digit span

The digit span test is based on the WAIS-III subtest using only the forward learning part of the test. It was used in this study as a general measure of attention at baseline and to acclimate subjects to the testing environment prior to performing the paired associates task. Numbers in this task are presented serially on a 15" VGA monitor for one second each followed by a one second ISI. After the numbers are presented subjects write down on a response sheet the numbers in the order they were presented. The first test series consists of three numbers with each series thereafter increasing by one number until the last series, which contains ten numbers. After responding to the last series of ten numbers, subjects then complete a second trial starting again with three numbers eventually increasing to ten numbers, this time using different number sequences. Digit span always immediately precedes the paired associates task. The same task is administered at retest using different number series. Performance is measured as the number of correctly recalled number series at baseline and retest.

Results

Pre-sleep variables

The half-night and full night sleep groups performed similarly on the Verbal and Performance scales of the MAB-II, and Full Scale IQs for both groups were also similar (see Table 5). Sleep log data for the three nights prior to the overnight session revealed no differences between the half-night and full-night sleep groups for time awake prior to the learning session at 10:45pm (½ night 14.7 ± 0.44, full night 14.0 ± 0.44 (mean ± SEM), $t_{(22)}$=1.06, p=.30) and average total sleep time for the three nights prior to the day

of the study (7.5 ± 0.34, 7.4 ± 0.32, $t_{(22)}$=.25, p=.80). (Means/SEMs for the ½ night sleep group are always presented first). Sleepiness prior to testing was also similar between groups (Stanford Sleepiness Scale (SSS): 3.2 ± 0.31, 2.8 ± 0.23, $t_{(22)}$=1.20, p=.24; Sleep onset latency (SOL): 16.0 ± 6.3, 18.4 ± 3.9, $t_{(22)}$=.33, p=.74). General sleepiness, assessed by the Epworth Sleepiness Scale, was similar between groups (7.8 ± .91, 6.8 ± .65, $t_{(22)}$=.96, p=.35) as was general motivation level, assessed by the Student Opinion Scale, (p>.3).

Sleep Data

Due to corrupted EEG data, sleep data for one subject were not included in the sleep analyses. A comparison of relevant sleep variables for the ½ night and full night sleep groups is presented in Table 6. The average total sleep time (TST) for the ½ night group was approximately 3.5 hours, while the full night sleep group obtained nearly 7.5 hours of sleep. In keeping with previous studies, subjects in the ½ night sleep group obtained a large proportion of SWS (31.2%) compared to REM sleep (9.7%).

Paired Associates

Overall performance. Performance on the semantically related paired associates prior to sleep was similar between groups (21.3 ± 1.9, 20.6 ± 2.2, $t_{(22)}$=.31, p=.22). Both groups showed a significant increase in number of paired associates recalled during cued recall at retest (paired samples t-test: ½ night, $t_{(10)}$=5.8, p<.001; full night, $t_{(12)}$=6.7, p<.001). Overall improvement after sleep was also very similar between groups for raw score improvement (Number recalled at retest – baseline learning: 8.3 ± 1.4, 8.6 ± 1.3;

sleep group x time interaction, $F_{(1,22)}$=.03, p=.86) (Figure 9) and percentage improvement over pre-sleep learning (45.3 ± 9.0, 53.2 ± 11.2, $t_{(22)}$=.54, p=.60). Correlations between all sleep parameters and performance improvement were non-significant.

Intelligence. Encoding of paired associates correlated with Verbal (r=.41, p=.05), Performance (r=.45, p=.03), and Full Scale IQ (r=.47, p=.02). Cued recall of paired associates at retest was also related to Verbal (r=.49, p=.02) and Full Scale IQ (r=.44, p=.03; Table 7). However, improvement in recall was did not correlate with IQ measures for either group (all p values<.17).

Number Sequence Learning

Overall performance. Prior to sleep, average time per trial (speed) (29.8 ± 2.1, 29.3 ± 1.5, $t_{(22)}$=.23, p=.82) and accuracy (3.0 ± 0.7, 2.2 ± 0.4, $t_{(22)}$=1.0, p=.32) was similar between groups. Both groups demonstrated a significant improvement in speed (paired samples t-test: ½ night, $t_{(10)}$=5.4, p<.001; full night, $t_{(12)}$=4.7, p<.001) following sleep. The ½ night sleep group showed improved accuracy following sleep (paired samples t-test: $t_{(10)}$=2.2, p=.05), while the full night sleep group showed a non-significant improvement at retest (paired samples t-test: $t_{(12)}$=, p=.22). Improvement after sleep was similar between groups for improvement in speed per trial (5.6 ± 1.0 sec, 5.8 ± 1.2 sec, $t_{(22)}$=.32, p=.75) and percentage improvement in speed (18.3 ± 3.0%, 19.2 ± 3.7%, $t_{(22)}$=.19, p=.85) (Figure 10). Improvement in accuracy was also similar between groups (1.7 ± .8, 0.8 ± 0.7, $t_{(22)}$=.86, p=.40; Figure 11). The only sleep parameters to correlate with improvement in speed were amount of stage 2 sleep (r=.66, p=.01; Figure 12), SOL (r=-.69, r=.009), and wake time (r=-.53, p=.06) in the 8-hour sleep group. Improvement

in accuracy correlated negatively with SWS for the total sample (r=-.44, p=.05) and marginally for the ½ night sleep group (r=-.57, p=.07).

Intelligence. Speed on the number sequence learning task prior to sleep marginally correlated with Full Scale IQ (r=-.33, r=.12). At retest, speed correlated with Performance IQ (r=-.42, p=.04) and Full Scale IQ (r=-.38, p=.06; Table 7). Improvement in speed after sleep was not significant for either sleep group (all p values>.5). Encoding accuracy, retest accuracy, and change in accuracy did not correlate with IQ scores.

Digit Span

Half night and full night sleep subjects recalled a similar number of number sequences prior to sleep (4.8 ± .43, 4.3 ± .43, $t_{(22)}$=.90, p=.38). While performance after sleep was stable in the ½ night sleep group, the full night group showed a slight improvement over pre-sleep performance. However, the sleep group x time interaction was not significant ($F_{(1,22)}$=2.2, p=.15). None of the sleep parameters correlated with improvement on the digit span task.

Intelligence. Full scale IQ correlated with performance on the digit span performance prior to sleep (r=.48, p=.02) and after sleep (r=.44, p=.03), but not with performance improvement after sleep (all p values>.3; Table 7).

Discussion

The present study set out to address two central issues. The first was to assess the effect of different durations of sleep on processing of declarative and motor memory

tasks. In addition to sleep duration, we were also interested in subject intelligence and how it relates to encoding facility and performance changes following sleep.

For both the semantically related paired associates and the number sequence learning task, subjects from both groups showed strikingly similar improvement after sleep, demonstrating that, while performance improves following sleep, the degree of improvement does not depend on obtaining larger amounts of nocturnal sleep. Interestingly, the paired associates improvement demonstrated by both groups in this study (8.3 for the ½ night sleep group and 8.6 for the full night sleep group) was comparable to the 8.75 improvement observed in subjects that took a short daytime nap (Tucker et al., 2006). For the motor sequence learning task, again we found very similar levels of improvement measured by reduction in time per trial at retest (17.0% for the ½ night sleep group and 18.6% for the full night sleep group). This percentage improvement is very similar to findings from a recent nap study that showed a 16% improvement in speed on an almost identical number sequence learning task (Nishida & Walker, 2006), which suggests that while sleep is important for processing of memories from multiple domains, greater amounts of sleep are not required for optimal performance. Interestingly, this pattern of findings also generalizes to perceptual learning tasks. It was shown that visual texture discrimination performance following a 90-minute daytime nap containing REM sleep was equivalent to the performance gains observed after a full night of sleep (Mednick et al., 2003).

What remains to be seen is whether the mnemonic benefit of a short period of sleep results in lasting changes in memory performance compared to longer periods of sleep. It may be that longer periods of sleep impart longer term benefits or, alternatively,

it may be that short bouts of daytime sleep, by virtue of unspecified circadian factors or EEG spectral characteristics, are all that are necessary to sustain the memory trace for longer periods of time. Clearly, there is ample reason to delve deeper into differences between daytime and nocturnal sleep to identify the physiological basis for these performance similarities.

It should be noted that, in keeping with the findings from Nishida & Walker (2006) and other studies demonstrating a relationship between amount of stage 2 sleep and motor memory (Walker, et al., 2003), we found that amount of stage 2 sleep in the full night sleep group was the only sleep variable to correlate with performance improvements following sleep on the number sequence learning task. While the underlying mechanisms have not yet been fully delineated, it seems likely that spindle activity, which is most prominently exhibited during stage 2 sleep, when expressed over longer periods of sleep could have a more beneficial effect on post-sleep performance. This idea fits well with theoretical accounts of how periodic spindle bursts occurring during stage 2 sleep could represent a mechanism by which experience-dependent plastic changes in neocortical circuits might be achieved (Sejnowski & Destexhe, 2000).

In a recent daytime nap study it was shown, using three declarative memory tasks, that the effect of sleep on memory was pronounced only if subjects had strongly encoded the information prior to sleep (Tucker & Fishbein, submitted for publication). This finding led us to hypothesize that specific subject variables may contribute to this strengthened encoding, which in turn would lead to a greater more dramatic effect of sleep. In the present study we focused on intelligence as a potential subject variable that might explain this effect. Interestingly, intelligence was generally predictive of

heightened information encoding and memory performance after sleep for all three learning tasks (including digit span), but intelligence did not predict a greater degree of improvement following sleep. Therefore, while we were able to isolate a subject variable that produced enhanced information encoding, this particular encoding boost did not translate to greater sleep-related gains as observed in our previous nap study. This finding, however, should not preclude further analysis of other variables that might more strongly predict this type of sleep-related memory improvement. For example, if we look at the problem from an evolutionary standpoint, the effect of a variable such as stimulus reward value may better elucidate the nature of memory enhancement following stronger encoding. Most studies thus far have only looked at performance on tasks in which the stimuli are of little value to subjects. Therefore, there is little reason to assume that subjects would be motivated to strongly encode or retain the information beyond the period of the study. If sleep has evolved to function in the service of memory consolidation, then it should be that when the rewards for learning and memory are greater (e.g., when performance results in a grade or a monetary reward), not only would we expect to see stronger encoding, but it may also be that sleep more strongly consolidates this type of information. Only two studies to our knowledge have examined a learning situation in which subjects were graded on performance. One study found that college students' REM sleep time and REM density increased following final exams (Smith & Lapp, 1991), and another showed that foreign language learning ability correlated with percentage of REM sleep over an intensive 6-week learning period (De Koninck et al., 1989). Both studies show that when subjects learn material that carries with it significant extrinsic reward value, interesting changes in relevant sleep parameters

are observed. Only one study examining the effect of sleep on memory has included a monetary reward for correct recall (Fischer, Drosopoulos, Tsen, & Born, 2006). However, reward per se was not manipulated in any of these three studies, but likely served to ensure heightened motivation from all subjects.

Overall, this study draws into question the idea that large amounts of sleep are necessary for optimal sleep-related memory gains. The findings also suggest that, while intelligence may not be a subject variable that modulates the effect of sleep on performance, future research should attempt to identify subject variables and task stimulus properties (e.g., stimulus reward value) that not only enhance encoding, but also lead to more pronounced performance improvements following sleep.

V

CONCLUSIONS

The study of the effect of sleep on memory has a long and rich history that clearly establishes it as more than a flash-in-the-pan endeavor. Indeed, over the many decades of rigorous scientific study of this topic researchers have certainly experienced more than a fleeting notion that understanding the nature of sleep-dependent memory processing would signify more than the mere unveiling of another benefit of sleep, and that one of the evolutionary purposes of sleep might be to consolidate information learned during wakefulness.

However, while there have been a number of groundbreaking and seemingly conclusive findings over the decades suggesting that sleep is necessary for optimal memory processing, it is clearly a notion that remains dubious in the eyes of many notable scientists who have generated a number of well-reasoned criticisms of the research (Vertes & Eastman, 2000; Siegel, 2001; Vertes & Siegel, 2005; Horne & McGrath, 1984). Luckily, with each salvo of criticism researchers have countered with creative solutions in theory and design that have endowed subsequent findings with greater significance and explanatory power. The fact that new concerns continue to surface over the years should not be taken as a sign that the field has reached a cul-de-sac, as has happened periodically over the decades. Instead, it should be an indication of the evolving nature of good science. Fortunately, within the last ten years a broad multidisciplinary effort has brought about a greater understanding of the consolidating properties of sleep and with it much greater resistance to opposition.

However, given the present strength of research findings over the past decade, it is still notable that there exist considerable difficulties regarding how best to interpret many of the findings. In the three studies presented here, we show that the effect of sleep on memory depends on a number of factors, including the memory domain tested, the semantic quality of the stimuli, the strength of information encoding, the manner in which stimuli were encoded, and the duration of the intervening sleep period. While these studies reveal a number of interesting effects of sleep on memory processing, they also raise a number of questions that future research should attempt to resolve.

In the first nap study, we extended the findings of Plihal & Born (1997) using a design that addressed several shortcomings of that study, and we found that even brief amounts of NREM sleep could produce improved recall compared to an equivalent period of wakefulness. While these results solidly favor NREM sleep as the basis for the improvement, one must consider alternative explanations. The only alternative explanation that appears reasonable is that the enhanced performance of the nap group was not so much an effect of sleep as a result of increased interference in the wake group. While this explanation seems unlikely, as both groups experienced similar amounts of wakefulness during the learning-retest interval (5 versus 4 hours), the effect of waking interference cannot be ruled out. Future research should strive to employ designs that effectively reduce the potential effects of waking interference, even though this has been a major challenge in the field thus far.

The second nap study extended the findings of the first study by using declarative memory tasks that varied in semantic quality. We found that paired associates performance benefited from a daytime nap containing only NREM sleep, but that

performance on the non-semantic tasks, while superior to wake subject performance, did not reach statistical significance. This finding leads to a number of interesting interpretations. First, it could be taken to mean that the benefits of NREM sleep do not generalize beyond the verbal/semantic domain, which would weaken the argument that hippocampus-dependent tasks in general benefit from NREM sleep. However, there are two reasons to forego such an argument. First, the amount of sleep obtained by subjects during the daytime nap was minimal (approximately 45 minutes). Longer durations of sleep (e.g., obtained during nocturnal periods) could reveal more dramatic differences in performance between sleep and wake groups. Another plausible explanation lies in the nature of the tasks. While the maze learning task and the complex figure task clearly rely on hippocampal mechanisms for initial processing, they also embody a procedural component that is not easily dissociable from the declarative. Therefore, given the known dependence on REM sleep for procedural memory processing, it may be that periods of sleep containing REM sleep (e.g., during a longer daytime nap) may more effectively differentiate sleep from wake performance.

Regarding paired associates performance it is noteworthy that nap subjects only improved more than wake subjects if they were immediately tested on the word pairs during the learning period, which replicates a number of previous findings. This leads one tentatively to conclude that there is something about this encoding process that enables sleep to more powerfully process the memory trace. The determining factor may have been encoding "strength," as it was shown with all three tasks that stronger encoding led to significant sleep-dependent improvements in performance. For paired associates specifically, it could also be that immediate testing on the word pairs during

the learning session provides the activation of cortical and hippocampal ensembles necessary to enable the hippocampo-neocortical dialogue to be more fully engaged during sleep. Each of these interpretations appears viable and in need of further empirical study.

The overnight study was noteworthy on several levels. For the first time it was shown that improvement for both declarative and motor learning task performance after a half night of sleep was the same as after an entire night of sleep, and that the magnitude of improvement on each of these tasks was very similar to that observed in previous studies examining performance following a daytime nap. One obvious conclusion is that only brief amounts of sleep are necessary to produce performance enhancements. However, it is possible that daytime naps do not confer the same memory benefits as longer durations of sleep over longer time intervals (e.g., a week or more after initial encoding). It is also possible that even though NREM sleep is clearly identified polysomnographically (based on a visual analysis of the EEG), there may be aspects of the EEG that contribute to memory processing that are not visually perceptible, namely the spectral characteristics of the NREM EEG waveform. A dissection of the sleep EEG via spectral analysis may go a long way to explain why so little sleep (obtained during the diurnal phase) could produce findings similar to those observed after longer nocturnal sleep opportunities.

The second major focus of the third study was to examine the modulatory influence of the subject variable intelligence on sleep-dependent memory processing. We reasoned that if stronger stimulus encoding leads to greater sleep-dependent improvements, then delimiting a group of subjects that encode well (i.e., intelligent

subjects) may allow us to tap a variable that plays a central role in the modulation of the sleep effect. While intelligence consistently correlated with information encoding and retrieval facility, it did not correlate with greater sleep-related improvements. Combining these findings with those from the second nap study, we can begin to see the importance, at this early stage, of addressing two potentially important modulating factors of the sleep effect: encoding strength and subject attributes thought to be tied to heightened information encoding and, therefore, greater sleep-related memory enhancement.

As with any well-studied scientific hypothesis, until alternative explanations for the findings are no longer forthcoming, the sleep/memory research community must willingly expect and accept continued criticisms of the research. Fortunately, even though the findings sometimes appear only to unmask the complexities of the topic, as opposed to resolving existing issues regarding the benefits of sleep for memory processing, there is ample evidence that real solutions to these issues are on the horizon.

VI

TABLES

Table 1. Sleep Parameters

	Minutes ± SEM	% of TST ± SEM
SL	11.75 ± 2.50	
TST	47.00 ± 4.13	
WASO	8.92 ± 3.13	
S1	5.17 ± 1.05	11.00% ± 2.30%
S2	19.38 ± 1.89	41.23% ± 4.66%
S3	9.63 ± 1.85	20.50% ± 3.69%
S4	12.80 ± 4.17	27.23% ± 6.66%
SWS (S3+S4)	22.43 ± 4.60	47.73% ± 6.65%

Note. SL-latency to sleep onset (first epoch of sleep); TST-total sleep time; WASO-wake time after sleep onset; S1-S4-stages 1-4; SWS-slow wave sleep

Table 2. Pre-study Variables

	Nap	Wake	t_{27}	p
Wake prior to learning	4.3 ± .56	4.0 ± .33	.50	.62
TST night before study	7.6 ± .31	7.5 ± .37	.26	.80
Mean TST 3 nights prior to study	7.3 ± .42	7.2 ± .35	.13	.89

Note. Time was measured in hours ± SEM; TST-total sleep time.

Table 3. Sleep Parameters.

	Minutes ± SEM	% of TST ± SEM
SL	14.97 ± 2.92	
TST	48.28 ± 3.49	
WASO	5.66 ± 2.33	
S1	4.78 ± 0.89	10.63 ± 1.96
S2	21.28 ± 1.61	46.19 ± 3.28
S3	14.13 ± 1.76	29.81 ± 2.84
S4	7.34 ± 2.77	13.36 ± 4.39
SWS (S3+S4)	21.47 ± 3.23	43.17 ± 4.03

Note. SL-latency to sleep onset (first epoch of sleep); TST-total sleep time; WASO-wake time after sleep onset; S1-S4-stages 1-4; SWS-slow wave sleep

Table 4. Pre-study Variables

	Nap	Wake	t_{31}	p
Wake prior to learning	4.9	4.6	.74	.47
TST night before study	7.1	7.6	1.1	.28
Mean TST 3 nights prior to study	7.1	7.6	1.4	.17

Note. Time was measured in hours ± SEM; TST-total sleep time.

Table 5. IQ scores for the ½ night and full night sleep groups.

	Sleep Condition			
	½ Night Sleep Group	Full Night Sleep Group	t(22)	p
Verbal IQ	103.7 ± 3.9	97.0 ± 3.4	1.3	.20
Performance IQ	99.8 ± 4.3	102.1 ± 3.1	.44	.67
Full Scale IQ	102.1 ± 4.1	99.5 ± 3.2	.50	.62

Note. IQ values are presented as means ± SEMs.

Table 6. Sleep Parameters.

	Sleep Condition			
	½ Night Sleep Group		Full Night Sleep Group	
	Minutes ± SD	% of TST ± SD	Minutes ± SD	% of TST ± SD
TST	211.5 ± 19.4		449.4 ± 27.1	
SE	89.0 ± 9.0		92.2 ± 4.0	
SL	16.0 ± 21.1		18.2 ± 14.8	
WASO	7.8 ± 8.9		19.2 ± 10.4	
S1	12.0 ± 4.1	5.8 ± 2.4	29.3 ± 9.6	6.5 ± 2.0
S2	111.9 ± 17.9	53.3 ± 9.3	231.3 ± 15.7	51.5 ± 3.4
S3	33.2 ± 19.0	15.5 ± 8.4	49.4 ± 15.2	11.0 ± 3.4
S4	33.4 ± 24.1	15.7 ± 11.0	24.4 ± 17.8	5.4 ± 3.9
SWS (S3+S4)	66.6 ± 21.2	31.2 ± 8.8	73.8 ± 16.4	16.4 ± 3.6
REM	20.5 ± 11.5	9.7 ± 5.1	115.1 ± 21.8	25.5 ± 4.1

Note. SL-latency to sleep onset (first epoch of sleep); TST-total sleep time; WASO-wake after sleep onset; S1-S4-stages 1-4; SWS-slow wave sleep; REM-rapid eye movement sleep; Spindle density-# of spindles from S2/S2 time (minutes)

Table 7. Correlations between IQ and task performance.

	Digit Span				Paired Associates				Number Sequence Learning			
	Learning		Retest		Learning		Retest		Learning		Retest	
	r	p	r	p	r	p	r	p	r	p	r	p
Verbal					.41	.05	.49	.02				
Performance					.45	.03					.42	.04
Full Scale	.48	.02	.44	.03	.47	.02	.44	.03	.33	.12	.38	.06

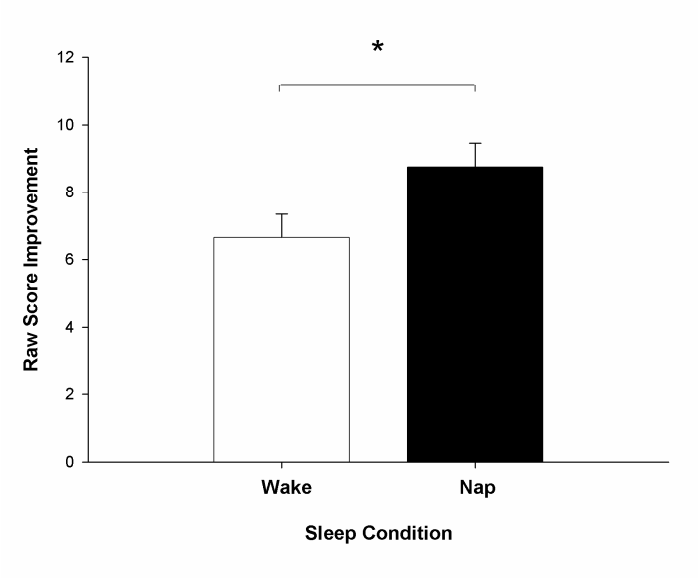
Note: Pearson product moment correlations (r) and their corresponding p values. Empty cells represent non-significant correlations. All correlations between IQ and performance improvement following sleep were non-significant.

VII

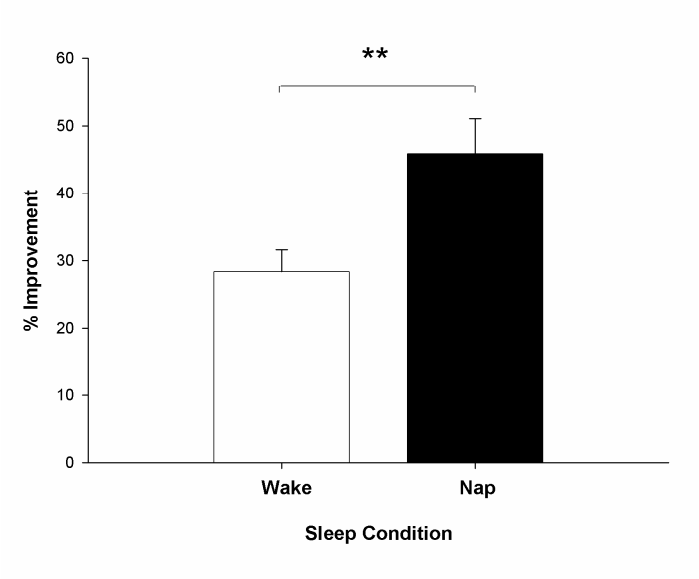
FIGURES

Figure 1.

a.



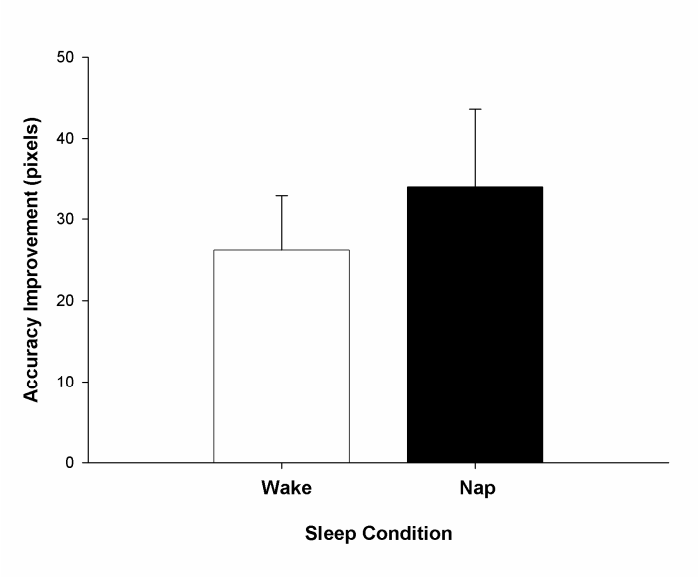
b.



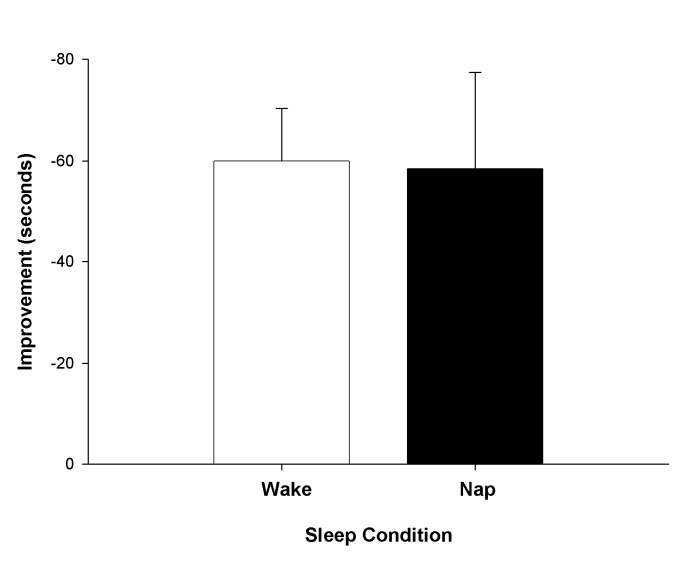
Improvement in paired associates performance. (a) Improvement is represented as the difference between number of word pairs correctly recalled at baseline training and retest six hours later. (b) Percentage improvement was calculated as raw number improvement from baseline to retest divided by baseline number correct. Values are presented as means ± SEM. A single asterisk represents a significant between groups difference at $p<.05$; double asterisk, $p<.01$.

Figure 2.

a.



b.

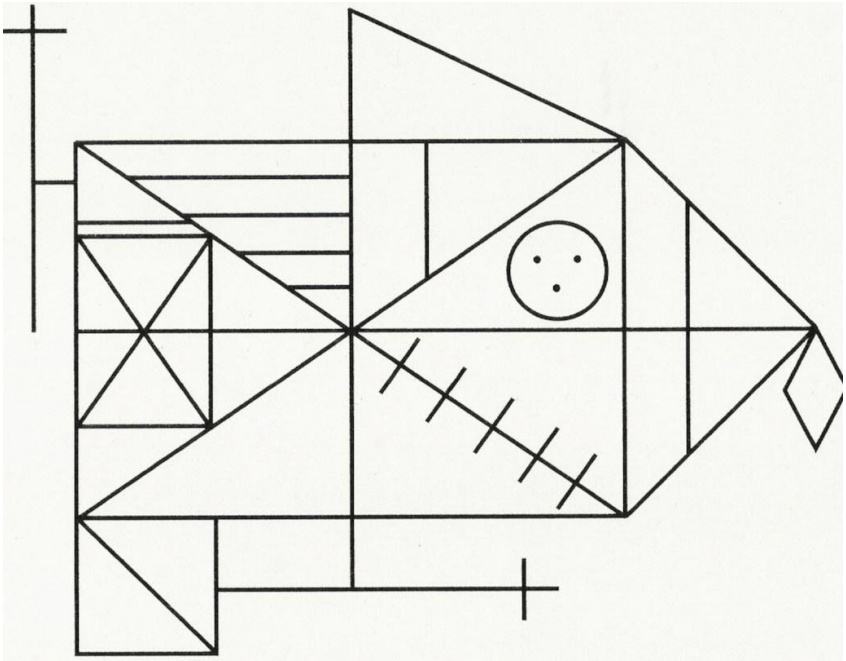


Improvement in mirror tracing performance. (a) Reduction in time was calculated as average time to complete the star tracings at retest minus average time at baseline. (b) Improvement in accuracy was calculated as average amount (number of pixels) drawn outside the star's boundary at baseline minus retest. Due to a positive skew pixel count was square root transformed. The transformed data are presented in the figure. Values are presented as means ± SEM.

Figure 3.

(a) Example of a mirror tracing trial from one subject. (b) Example of the same mirror tracing star after masking of all marks other than those lying outside the perimeter of the star's boundary

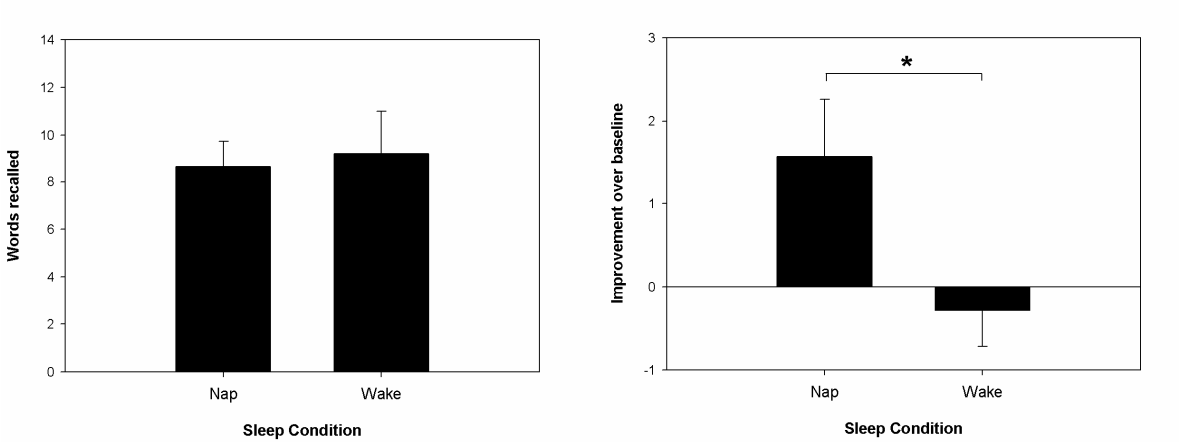
Figure 4.



The Rey-Osterrieth Complex Figure.

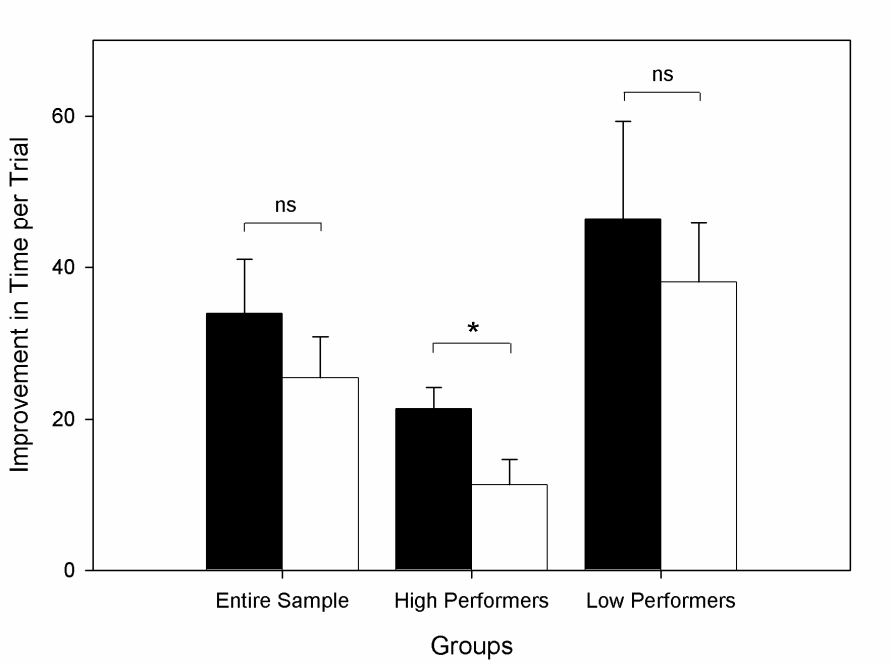
Figure 5.

a. b.



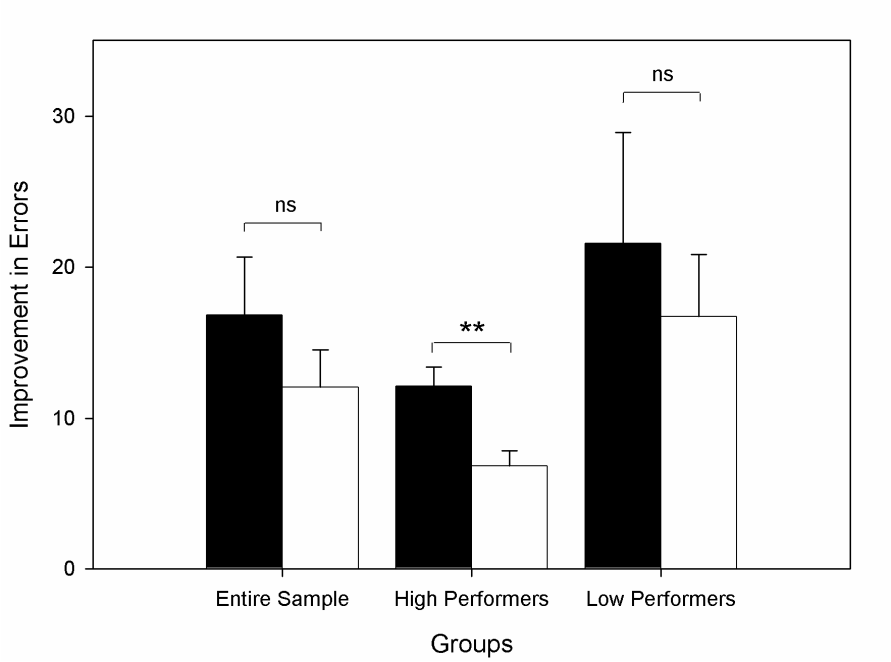
Unrelated paired associates performance. a) Recall of studied words at retest. b) Performance change (number of word pairs recalled at retest – recall at retest) for tested word pairs. Bars represent means ± SEM. * $p<.05$.

Figure 6.



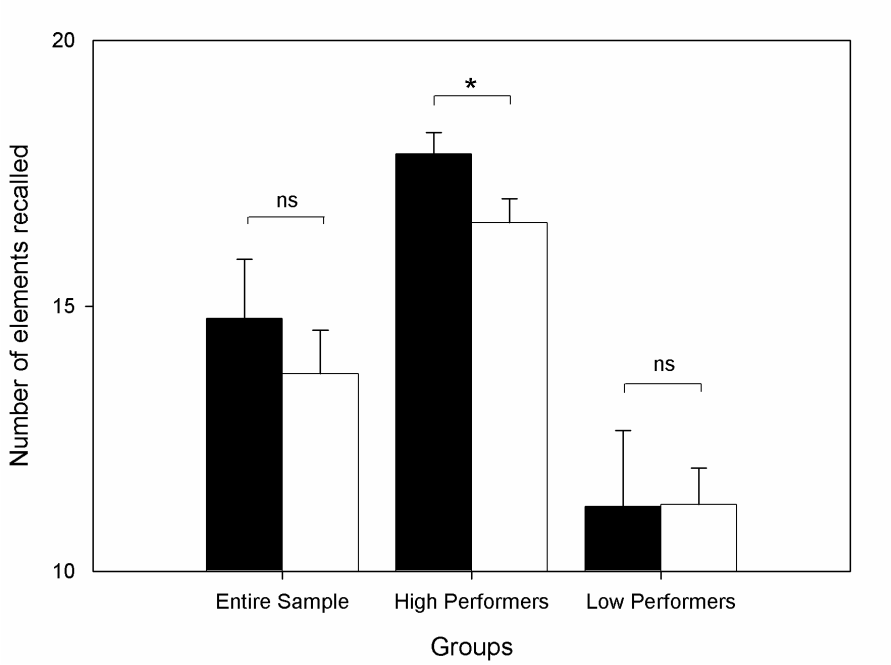
Differences between nap and wake groups for number of errors committed on the maze task. Performance change was measured as number of errors committed at baseline – number of errors at retest. Results (means ± SEM) are presented for the entire sample, high performers, and low performers. Black bars – Nap subjects; White bars – Wake group. ** p<.01.

Figure 7.



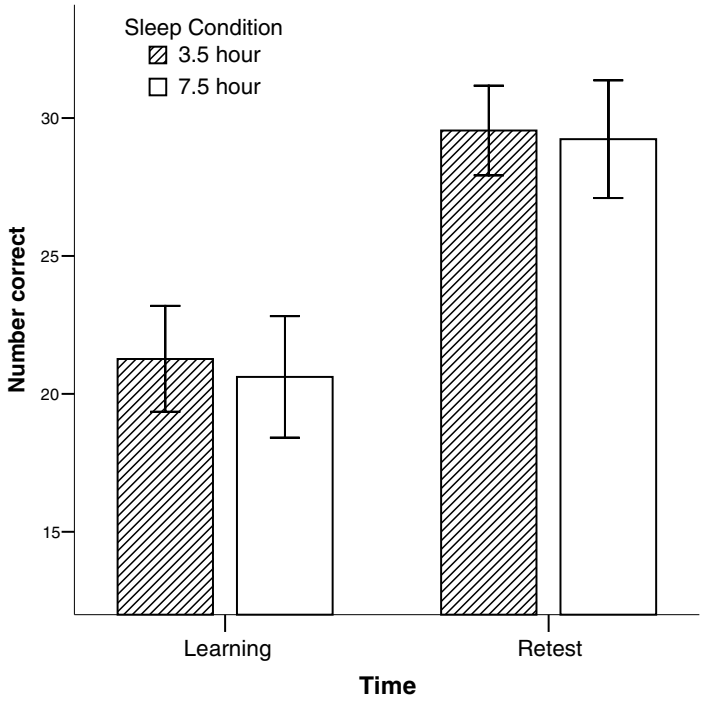
Differences between nap and wake groups for improvement in speed on the maze task. Performance change was measured as average time per trial at baseline – average time at retest. Results (means ± SEM) are presented for the entire sample, high performers, and low performers. Black bars – Nap subjects; White bars – Wake group. * $p<.05$.

Figure 8.



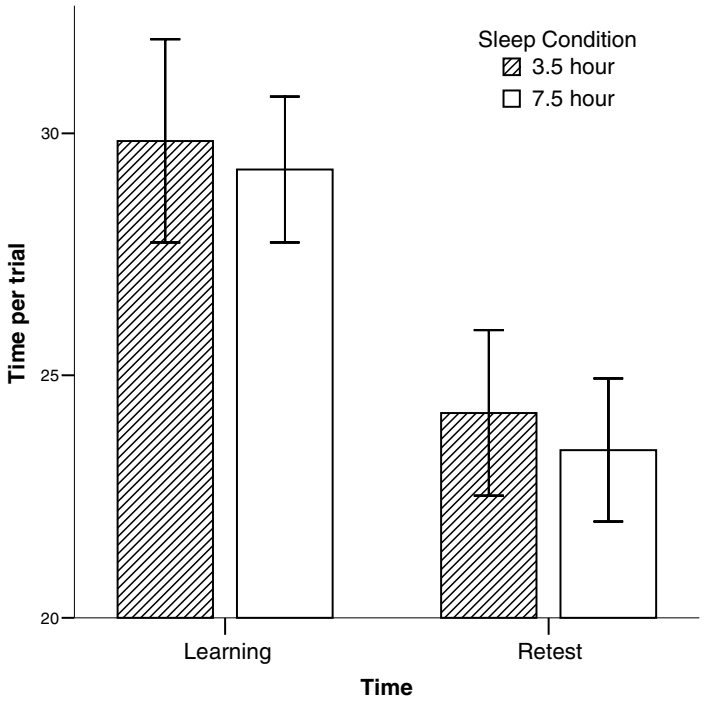
Differences between nap and wake groups for the Rey-Osterrieth complex figure task. Performance was measured as number of correctly recalled figure elements at retest. Results (means ± SEM) are presented for the entire sample, high performers, and low performers. Black bars – Nap subjects; White bars – Wake group. * p=05.

Figure 9.



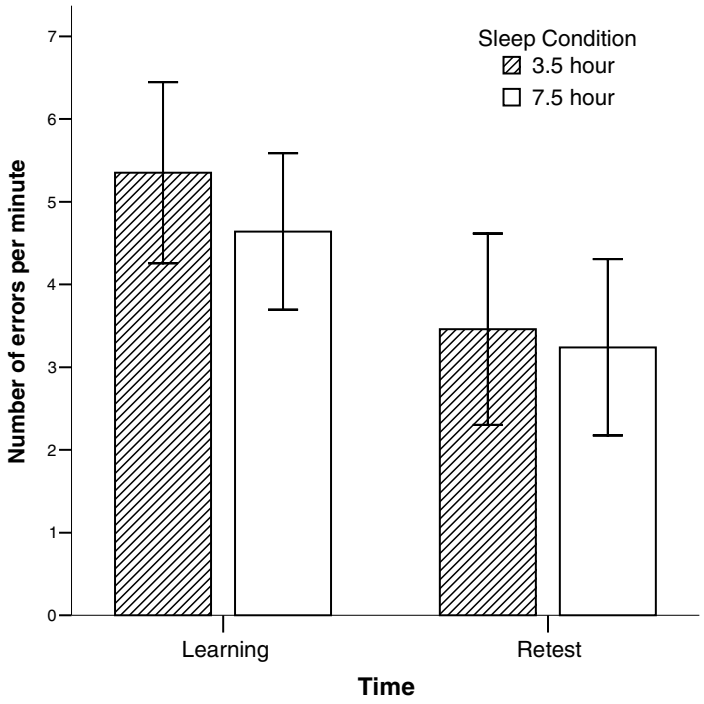
Paired associate performance immediately after learning and at retest either 3.5 or 7.5 hours into the sleep period. Performance is measured as number of correctly recalled word pairs.

Figure 10.



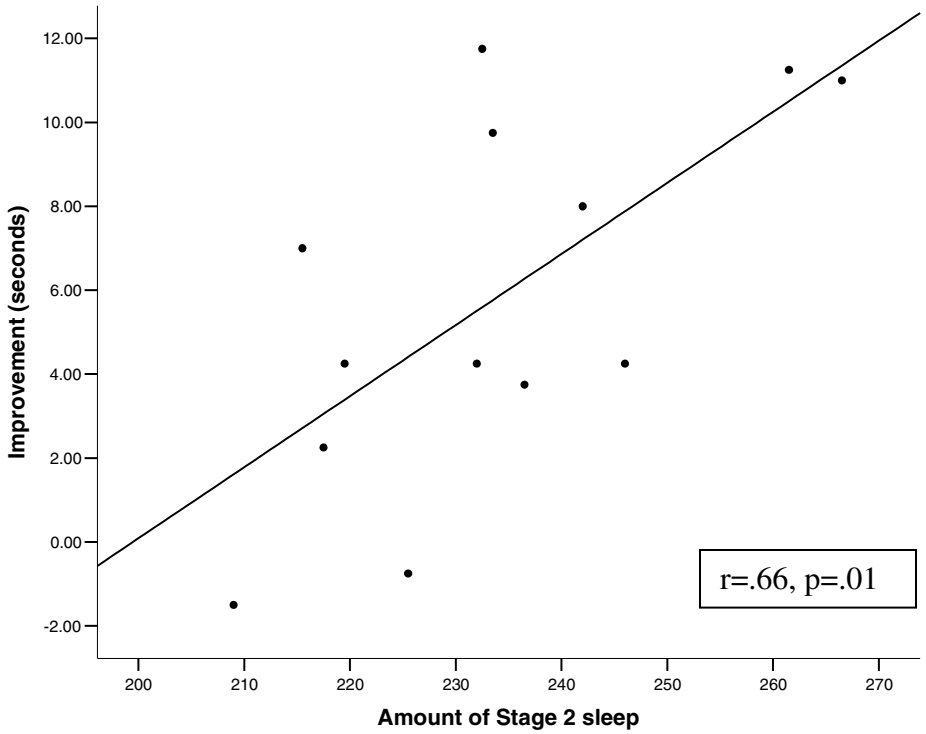
Average time per trial on the number sequence learning task immediately after learning and at retest either 3.5 or 7.5 hours into the sleep period. Performance is measured as average speed (seconds) to complete each 20-sequence trial.

Figure 11.



Accuracy on the number sequence learning task immediately after learning and at retest either 3.5 or 7.5 hours into the sleep period. Accuracy is measured as average number of errors per minute for each 20-sequence trial.

Figure 12.



Correlation between amount of stage 2 sleep in the full night sleep group and improvement (speed) on the number sequence learning task.

VIII

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