The Temporal Relationship between Daytime Napping and Memory Consolidation

by

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Abstract

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An extensive body of literature exists substantiating the idea that sleep facilitates the strengthening, stabilization, and protection of newly formed memories, aiding in consolidation from short-term to long-term stores. However, research as to the temporal boundaries of the benefit of sleep to declarative memory is deficient. It has been established that sleep benefits memory compared to equal time spent awake, but *when* sleep needs to occur relative to the learning period, as well as *how much* and *what type* of sleep is necessary, has been little explored. Additionally, researchers have focused on how the brain works on previously encoded information during sleep, but very few have addressed whether sleep prepares the brain to take on new information when it occurs *prior* to learning. Using efficient daytime naps, the present series of studies addressed these shortcomings and the results provided support exclusively to an *active* role for sleep in memory processing. Study I unexpectedly demonstrated superior performance for recognition memory with increased delay before sleep onset, resulting in increased slow wave sleep (SWS) in the later nap groups. Study II determined that sleep must progress into SWS, rather than merely Stages 1 and 2, for better short-term retention, subsequent protection from stimulus-related interference, and long-term consolidation, although even a brief nap provides temporary retention benefits over remaining awake. Examining

sleep *prior* to learning in Study III, it was found that a 60-minute nap prepared the brain to more efficiently consolidate information, despite the fact that nap and wake groups encoded material equally. Overall, the present research provides clarification, although perhaps task-dependent, to the existing questions regarding the temporal relationship between sleep and learning. Additionally, the results proffer support for active processing during sleep potentially through standard consolidation and/or homeostatic downscaling of synaptic potentials, the major mechanistic theories ascribing a role for SWS in declarative memory processing.

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Overall Introduction

I. Sleep Facilitates Memory Consolidation

There is a substantial body of evidence supporting the idea that sleep facilitates the consolidation of both procedural and declarative memories (Ekstrand, 1967; Plihal & Born, 1997; Smith, 2001; Walker & Stickgold, 2004; Stickgold, 2005; Born et al., 2006). Many behavioral studies demonstrate that a period of learning followed by sleep, as opposed to an equal time spent awake, benefits performance on a variety of tasks designed to measure different types of memories in animals, such as object recognition tasks (Palchykova et al., 2006), spatial reference tasks (Youngblood et al., 1997, Smith & Rose, 1997), discriminative and passive avoidance tasks (Fishbein et al., 1971; Linden et al., 1974, Alvarenga et al., 2008; Silva et al., 2004), and fear extinction (Silvestri, 2008), as well as in humans (Yaroush et al., 1971; Fowler et al., 1973, Fischer et al., 2002; Walker et al., 2002; Mednick et al., 2003; Gais & Born, 2004a). Implicit, procedural memory has been seen to benefit from a subsequent period of sleep, generally showing improvement from baseline levels in tasks measuring perceptual learning (Karni et al., 1994; Stickgold et al., 2000; Gais et al., 2000) and motor skills (Maquet et al., 2003; Rasch et al., 2009) such as finger tapping (Fischer et al., 2002; Walker et al., 2002). Explicit, declarative memories, both episodic memories for events and experiences as well as semantic memories for facts, also benefit from sleep, showing either enhancement of learned material over sleep or a preservation from decay (Wagner et al., 2001; Pare et al., 2002; Mednick et al., 2003; Kali & Dayan, 2004; Tucker et al., 2006; Gais et al., 2008, Nishida et al., 2009), as well as lead to insight (Wagner et al.,

2004) and indirect relational memory (Preston et al., 2004; Ellenbogen et al., 2007; Lau et al., 2010).

Along with a myriad of behavioral studies, demonstrating that those who sleep perform better than those who remain awake, there are many physiological studies that use functional imaging, track cerebral blood flow, and record cellular firing activity, to confirm and further bolster this claim. Changes in sleep parameters during post-learning sleep, such as augmentation of REM sleep (Fishbein et al., 1974; Smith et al., 1980; Hennevin & Hars, 1987; Ambrosini et al., 1989; De Koninck et al., 1989; Sanford et al, 2001; Smith et al., 2004), increases in sleep spindle density (Gais et al., 2002; Clemens et al., 2005; Schabus et al., 2004, Eschenko et al., 2006; Fogel & Smith, 2006), and increases in slow wave activity in areas associated with the memory task (Huber et al., 2004), have been correlated with superior performance after sleep. Additionally, changes in hippocampal activation and involvement, such as increases in hippocampal activation (Peigneux et al., 2004), increases in human electroencephalographic coherence (Molle et al., 2004), and shifts in brain area activation (Orban et al., 2006; Takashima et al., 2006; Gais et al., 2007) demonstrate consolidation from short-term to long-term stores.

II. Passive, Permissive, or Active

While it is clear that with a period of sleep, memory is better retained through consolidation, demonstrating signs of stabilization and prevention of decay through strengthening of synaptic connections, it is still argued as to the nature of sleep's role in memory processing. As elegantly illustrated by Ellenbogen et al. (2006), the contributions of sleep to the consolidation of memories could potentially fall into one of

three categories, or may have no role whatsoever, although this last suggestion has fairly been eliminated with the abundance of evidence against it. The *passive* theory, as demonstrated by the early seminal research of Jenkins and Dallenbach (1924), states that memories benefit from a period of sleep due to the relative lack of interference present during this state, regardless of when this period occurs, compared to a waking state. In other words, there is nothing inherently special about sleep, but rather, it is merely that one is shut off from the influence of incoming information that protects the memory for the duration of sleep (Wixted, 2004; Vertes & Siegel, 2005). However, this theory asserts that memory will once again be susceptible to interference once sleep is terminated. The *permissive* hypothesis agrees that sleep provides a reduction of interference protecting the memory trace, but this period needs to occur within a window of time in which the memory is still labile and able to benefit from such off-line processing. According to this theory, sleep allows more efficient consolidation than if occurring while awake and susceptible to interference, although consolidation is happening regardless of the state. In this view, there is a time-dependent nature to consolidation in which memory is unstable for a certain amount of time post-learning, after which gene expression and synaptic modifications are complete and memory is stabilized. The sleep interval must occur during this window for maximum consolidation benefits, after which, sleep will have no effect. Conversely, the *active* hypothesis ascribes unique properties to the dynamics of sleep stages and inherent physiological phenomena, such as sleep spindles, sharp wave ripples, and pontine-waves, which facilitate memory processing in a way that cannot be achieved under other circumstances. The evidence discussed above regarding changes in hippocampal activation after sleep,

changes in spindle density with learning, local increases in slow-wave activity, shifts in brain activation after sleep, etc., supports this idea of sleep's active role in processing. The present studies adopt the *active* view and are uniquely designed to add support to this hypothesis.

III. Sleep Stages and Declarative Memory

The literature on sleep and memory is so prolific that it necessitates a narrow focus on hippocampal-dependent declarative memory, relatively more clearly mapped and understood, for the purpose of these studies. Encoding and storage of declarative memory is known to require both the cortex and the medial temporal lobe (MTL), specifically the hippocampus and surrounding areas, including entorhinal, perirhinal, and parahippocampal cortices (Eichenbaum, 2000). It begins with the rapid encoding of new information through waking interaction with the environment, gaining new experiences and exploring surroundings. Information instigates a flow of activation from sensory receptors, through thalamic weigh stations, with the exception of olfactory processing, then through the entorhinal cortex (EC) to the input layers of the CA3 region of the hippocampus, both directly and through the dentate gyrus (Chrobak & Buzsaki, 1994). This rapid encoding is facilitated by high acetylcholine levels in the brain, which suppress the glutamatergic synapses in the hippocampus, allowing the formation of a temporary representation of the memory in a labile state through a predominantly sensory cortex-to-MTL directional flow of information without interference from existing memory stores (Hasselmo, 1999).

While the newly encoded representation exists in the hippocampus, which links the cortical areas involved in the experience (Teyler & DiScenna, 1986), it is subject to forgetting unless further processing occurs. Studies of amnesiacs, like H.M., who have lost partial or total functioning of the hippocampus and surrounding areas, reveal a gradient time dependency of declarative memories on the MTL. Such a gradient allows older memories to be preserved in the neocortex, while newer memories are lost, without the hippocampus to connect the disparate cortical areas involved in the memory (Squire & Zola-Morgan, 1991; Scoville & Milner, 1957)(although see Piolino et al., 2009 for support that autobiographical episodic memory may continue to rely on the hippocampus). A certain degree of this "transfer" of dependence to the neocortex is thought to happen simply through time-dependent consolidation, independent of sleep (McGaugh, 2000). However, the unique interference-attenuated, neurochemically ideal state of sleep, specifically non-REM (NREM) sleep, is thought to best actively facilitate this consolidation process. As the brain passes from a waking state, through Stages 1 and 2 and into slow-wave sleep (SWS), glucocorticoids, which exert strong feedforward actions to the hippocampus and other limbic structures that are densely packed with receptors, are inhibited (Plihal & Born, 1997), greatly reducing the flow of information into the hippocampus. At the same time, acetylcholine levels drop, releasing the glutamatergic suppression creating a predominantly hippocampus-to-neocortex directional flow of information, an ideal setting for consolidation (Hounsgaard, 1978; Valentino & Dingledine, 1981; Rovira et al., 1983; Hasselmo et al., 1992; Herraras et al., 1988; Hasselmo, 1999; Gais & Born, 2004b; Hasselmo & McGaughy, 2004). Studies involving the manipulation of slow oscillations (Marshall et al., 2004; Marshall et al.,

2006; Kirov et al., 2009) or introduction of contextual learning cues during SWS (Rasch et al., 2007), have shown the direct connection between this sleep stage and declarative memory consolidation.

There are two major theoretical frameworks from which the majority of sleep and declarative memory research is derived, the complexities of which are important to understand in order to appreciate the *active* theory of the role of SWS. According to the standard two-stage theory of consolidation (Buzsaki et al., 1983; Buzsaki, 1989; McClelland et al., 1995), the first stage begins with rapid encoding during wake, as described above. Subsequently, during SWS, sharp wave ripples, or fast hippocampal neural oscillations (150-250 Hz), grouped by, and occurring in the transition between, the slow oscillations of SWS delta waves (.5-2 Hz) (Sirota et al., 2003; Battaglia et al., 2004), participate in the reactivation of neuronal networks that were most recently fired during waking, such as those representing declarative memory (Wilson & McNaughton, 1994; Louie & Wilson, 2001; Ji & Wilson, 2007). Best demonstrated by Wilson & McNaughton (1994), and confirmed by several other studies (Pavlides & Winson, 1989; Skaggs & McNaughton, 1996; Kudrimoti et al., 1999; Nadasdy et al., 1999), reactivation of waking neuronal activity was found to occur primarily during SWS. They examined neuronal firing patterns of place cells in the hippocampi of rats exploring an environment prior to sleep and observed the activity of the same neurons, in a time-compressed manner, in the same temporal order, during SWS (however, see Hennevin et al., 2007, for a critique). The conclusion to this observation was that replay led to long-term potentiation (LTP), the predominant candidate as a mechanistic explanation for synaptic consolidation within a network (Sirota et al., 2003; Brehens et al., 2005; Whitlock et al.,

2006; Diba & Buzsaki, 2007), resulting in strengthening of synapses due to gene expression and subsequent structural modification of the pre-and post-synaptic cells, leading to a gradual shift of the burden of reactivation from the hippocampus to long-term stores in the neocortex. The strength of the bursts of sharp wave ripples is sufficient to induce LTP within the network (Buzsaki, 1989) and recent evidence ascribes a causal role to sharp wave ripples in memory consolidation. Selective suppression of sharp wave ripples in rats trained on a spatial task prior to sleep resulted in significant performance impairments during retesting, although it has not been determined whether the absence of ripples or disruption of neuronal replay during sleep is the exact cause of the impairment (Girardeau et al., 2009; Ego-Stengel & Wilson, 2010).

An alternate, although not mutually exclusive, theory proposed by Tononi & Cirelli (Tononi & Cirelli, 2003, 2006) gives SWS an indirect role in memory processing. This theory is based on the observation that with long periods of use, through everyday activities but specifically with focused learning, synapses are continuously potentiated, depleting synaptic resources, increasing synaptic strength, and increasing the threshold for firing. Tied to this observation is an increase in the need for homeostatic regulation of SWS, so that the greater the need, the more SWS is seen at sleep onset, decreasing over the sleeping period. The theory states that SWS serves to globally downscale, or reduce synaptic weights, bringing them back to baseline levels and essentially preparing the brain to again be plastic and take on new information. This downscaling happens as a result of the alternating synchronous hyper- and depolarization that occurs during SWS, with the changes in membrane potential serving to "reset" the synaptic strengths. Local increases in slow wave activity have been shown to correlate with the location of

activation during intensive learning, so that SWS can locally and globally return the brain to baseline (Huber et al., 2004). Memory is essentially thought of as a by-product of this active process occurring during sleep. While there is global downscale of synaptic strength, the synapses involved in a learned memory trace are stronger than those involved in firing during everyday waking experience, so as the latter falls below threshold for firing, the former, the memory trace, stands out in an increase of the signal to noise ratio. However, it is unclear whether downscaling benefits memory by also occurring in the hippocampus proper, which has conflictingly been shown to maintain firing rate over time (Buzsaki et al. 2002), or only the cortical areas potentiated by learning and associated with the memory trace.

While these theories focus on SWS, the possible contribution of other sleep stages, such as Stage 2 and REM sleep, to the initiation, continuation, or completion of the memory processing seen during SWS cannot be ruled out. A few studies have demonstrated functional changes in Stage 2 sleep parameters that implicate this stage in declarative memory processing (Gais et al., 2002; Clemens et al., 2005; Schabus et al., 2004). In these experiments, subjects' baseline sleep spindle density, measured as the number of 12-16Hz bursts of activity within Stage 2 sleep per 30s epoch, increases after a period of intensive learning compared to non-learning activity. In this line of research, a correlation has been found demonstrating that only those showing increased spindle density during post-learning sleep show an increase in performance on memory tests (Schabus et al., 2004). Spindles have been temporally correlated with sharp-wave ripples in animals (Siapas & Wilson, 1998; Sirota et al., 2003) as well as slow oscillations (Steriade & Timofeev, 2003), and are thought to represent memory processing. More

recent work has divided sleep spindles into slow, centro-parietally located spindles (~ 12-14Hz) and fast, frontally located spindles (~14-16Hz), each with different generators and potentially different roles in memory consolidation, although the role of each of these is beyond the scope of the present research.

Additionally, there is a rich history of REM sleep's contribution to memory, although generally to procedural memory. However, there is growing evidence supporting REM sleep's role in emotional memory consolidation (Wagner et al., 2001, Hobson & Pace-Schott, 2002; Hu et al., 2006; Nishida et al., 2009, De Jesus et al., in preparation). It is unknown whether emotional memories, generally declarative in human studies, are processed by component, with SWS processing the declarative episodic component while REM sleep facilitates the emotional component, or all together. REM deprivation studies, however, do show impairment of emotional memories, even when only the second half of the night is deprived (De Jesús et al., submitted for publication). REM sleep has also been hypothesized to contribute to memory consolidation by working in conjunction with SWS. Stickgold et al. (2000) found that following a period of sleep lasting at least 6 hours, there was a strong positive correlation between performance and the amount of time spent in REM sleep in the last quarter of the night and SWS in the first quarter of night. They proposed a 2-step model: During SWS information flows out of the hippocampus to the neocortex replaying memories by reactivating the neuronal networks previously activated in waking experience. During REM sleep, hippocampal input to neocortex is inhibited, again through high levels of acetylcholine (Hasselmo, 1999), and neocortical networks are strengthened without hippocampal interference, gradually interleaving new information into preexisting long-term stores. They attributed

the importance of the 1st and 4th quartile to slower cellular processes, such as second messenger systems, cAMP response element binding (CREB)- dependent protein synthesis, and gene expression that requires time to complete. While a procedural task was used in this discovery, it nonetheless elucidates the need to consider the contributions of both kinds of sleep, which explains why the present studies did not systematically eliminate REM sleep and restrict the investigation to NREM alone like prior studies (Tucker et al., 2006).

IV. The Temporal Boundaries of Sleep's Benefit to Memory

The accumulated evidence that substantiates the idea that sleep facilitates the processing of newly formed memories is extensive, as has been discussed. However, research as to the temporal relationship between learning and sleep is lacking and is the focus of the present studies. It has been established that sleep benefits memory compared to equal time spent awake, but *when* sleep needs to occur relative to the learning period and *how much* and of *what type* of sleep is necessary has been little explored. Additionally, researchers have focused on how the brain works on previously encoded information during sleep, but very few have addressed whether sleep may prepare the brain to take on new information when it occurs *prior to* learning.

There has been little research as to the timing of post-learning sleep in relation to the learned experience. The majority of literature demonstrates that sleep must occur immediately after learning in order to benefit performance. However, a handful of recent studies find that sleep aids in the retention, or perhaps recovery, of memory even when it occurs after a substantial delay between learning and sleep onset (Fenn et al., 2003;

Backhaus et al., 2007; Rascmany et al., 2007). Performance on memory tests after delayed sleep has been shown in some cases to be similar or only slightly degraded from that seen when sleep immediately followed learning, with all sleep groups performing better than waking groups, implying an *active* role of sleep in memory processing.

Secondly, little research has been conducted to assess how much sleep is necessary to facilitate memory consolidation. While, it has been demonstrated that both a daytime nap, usually around 60-90 minutes, and overnight sleep benefits memory performance similarly, only one study (Lahl et al., 2008) demonstrated that an ultra-brief period of sleep, a 6-minute nap, had a beneficial effect on the retention of learned material. The fundamental question with this timing issue is whether or not the initiation of sleep is sufficient to begin the active processing of memory that persist over time or if stages of sleep achieved in a longer period of sleep, such as SWS, are necessary for consolidation to occur.

Lastly, while much is known about how post-learning sleep facilitates memory, very few recent studies (Axmacher et al., 2009; Nishida et al., 2009, Mander et al., 2011) have investigated how sleeping prior to learning prepares the brain to encode and process subsequent material. These studies have conflicting conclusions, partly due to the tasks and protocols employed in each study, and so the issue remains one that needs further exploration.

The present studies will address these specific shortcomings, with each of the three studies concentrating on one of these three areas of research. A more comprehensive review of the relevant literature concerning each topic will be included with each study.

V. The Daytime Nap Protocol

The use of a nap protocol in the series of studies here is intended to address inherent confounds that arise in two typical variations of overnight sleep designs. In one design, comparisons are conducted between overnight groups, those who sleep normally versus those who are deprived and remain awake, with results speaking more to the detrimental effects of deprivation rather than the processing of memory over a natural sleep period. Alternatively, overnight sleep groups are compared to daytime wake groups, with the possibility of circadian confounds interfering with the results, which can only be controlled to a certain degree using short-term retention morning and evening groups. Nap designs are increasingly used to practically address these issues and are of sufficient length to differentiate performance between sleep and wake groups (Mednick et al., 2003; Schabus et al., 2005; Backhaus & Junghanns, 2006; Tucker et al., 2006; Lau et al., 2010). Due to the length of an average nap when given a sleep opportunity of 60-90 minutes and the natural sleep architecture within this period, the predominance of NREM sleep aids in examination of declarative memory processing. Aside from the benefits of using this protocol in sleep research, there is also tremendous practical value in daytime nap research, in that naps are already a common occurrence in the greater population and may prove to lead to cognitive benefits with minimal effort for those in need of a learning edge.

VI. A Brief Summary of the Present Studies

The temporal relationship between learning and sleep remains unclear in several ways. The present series of studies addressed three major shortcomings in this area:

- *How soon does sleep need to occur in order to benefit memory?* While it is generally accepted that a period of sleep immediately following a learning session aids in the stabilization of the information encoded during learning, not much is known about the time-dependency of learning on sleep onset. Study I examined whether a nap benefited declarative memory when it occurred after a delay was imposed between learning and sleep, and examined the relationship between the magnitude of the delay and performance on a memory task.
- *How much sleep and what type of sleep needs to be achieved to stabilize and consolidate memory?* According to the predominant theories giving sleep and its many stages a role in memory consolidation, a myriad of studies support the idea that slow-wave sleep (SWS) is the ideal stage to promote the consolidation of declarative memories, either through active processes during sleep or as a byproduct of homeostatic regulation. However, researchers have not ruled out passive lack of interference during sleep or quiet wakefulness as an explanation for memory preservation. Study II addressed this distinction by comparing those who attained a nap containing SWS to those who remained awake or attained a truncated nap reaching no more than Stage 2 sleep. The effects on short-term recall, protection from interference, and long-term consolidation were examined.
- *Does sleep prepare the brain for new information?* Much of what is understood about the relationship between sleep and learning involves exploring the effects of post-learning sleep on subsequent memory task performance. However, very little

research has been conducted to understand whether a period of sleep *prior* to learning prepares the brain to encode information and facilitates future consolidation of that new material. Study III used a daytime nap to assess this issue and examined differences between those who slept and those who remained awake before learning in encoding efficiency, short-term retention, and long-term consolidation.

Study I: The effects of a staggered sleep onset paradigm on recognition and spatial memory

Introduction

There is an abundance of evidence supporting the idea that a period of learning is best facilitated by a subsequent interval containing sleep rather than wake. However, there is conflicting evidence as to when the period of sleep needs to occur in relation to completion of learning. The majority of studies examining sleep's influence on memory conclude that sleep should occur immediately after learning for optimal consolidation. The assumption here is that a memory trace decays over a waking period, with incoming interfering information competing with, and overriding, the learned material. Therefore, sleeping immediately after learning stabilizes and strengthens the synapses representing the memory and prevents subsequent decay, compared to remaining awake. According to this idea, the longer post-learning sleep onset is delayed, the less optimal consolidation will be, with the sleep period acting to consolidate the degraded memory and performance at testing inferior to that of those who slept immediately after learning. However, a few studies have demonstrated recovery of memory with sleep, with resulting performance at retest being similar for all sleep groups, regardless of the delay before sleep onset. These studies lend support to an *active* theory of sleep's role in memory consolidation in that they demonstrate memory facilitation unique to the state of sleep.

A 2003 study by Fenn et al. examined perceptual learning of synthetic spoken language, with subjects undergoing a baseline pre-test, followed by training on the task and a subsequent retention interval containing sleep/no-sleep, then post-test. They

compared a 12hr wake group (9am-9pm) to a 12hr sleep group (9pm-9am) and, as expected, found a significant difference in improvement, with the sleep group performing far better than the wake group. They also included two 24hr groups, both of which contained a period of sleep within the retention interval, although one (9am-9am) experienced a full day of wake before sleep onset, while the other (9pm-9pm) slept soon after learning. The authors discovered that both 24hr groups performed similarly to one another as well as to the 12hr sleep group (9pm-9am), all significantly better than the 12hr wake group (9am-9pm). These results led to the conclusion that sleep, regardless of when it occurs, actively stabilizes memory and prevents future decay (9pm-9am 12 hr group and 9pm-9pm 24 hr group) as well as recovers memory, seen to decay across the day (9am-9am 24hr group).

Another example of sleep's active role in consolidation is Backhaus et al.'s 2007 investigation of children who learned word pairs in two conditions. In one group (Sleep-Wake), subjects were trained on the word pairs in the evening, spent the night sleeping normally, and were tested in the morning and then retested in the evening on the second day. The second condition (Wake-Sleep) was opposite, with training in the morning, testing that evening, and retesting the following morning. In the Sleep-Wake condition, performance at test (after sleep) was significantly better than the Wake-Sleep group (after wake), and performance remained at the same level over the subsequent waking retention interval, measured at retest, showing how sleep actively stabilizes and protects the memory from later interference and decay. However, the poor performance in the Wake-Sleep group at testing was seen to *improve* at retesting after the subsequent overnight

sleep interval, demonstrating an active restorative role of sleep, recovering the degraded memory.

While these studies are intriguing, the body of literature showing an unrecoverable decay in memory when sleep is delayed causes confusion. The present study attempted to lend support to one idea or the other by examining the time-dependent relationship between learning and sleep using a staggered nap schedule, in which separate groups napped starting either immediately, 2-hours, or 4-hours after a period of learning and were compared to a group that remained awake for the duration of the experimental manipulation. A declarative visual recognition task was employed, in which subjects viewed neutral pictures of people, objects, and landscapes and were later tested on their ability to distinguish previously viewed from new pictures. To both examine spatial memory as well as add complexity and richness to the memory trace, a spatial aspect to the task was also included, which required participants to view the picture stimuli in one of four quadrants on the computer screen and later recall where the picture had appeared. It was hypothesized that a 90-minute nap, primarily comprised of NREM sleep, as compared to an equal period of wakefulness, would result in better performance at retest, compared to baseline measures, for both recognition as well as spatial memory. Based on differing evidence within the literature, the experiment was undertaken with competing hypotheses with regard to the temporal relationship between sleep and performance. One hypothesis, supported by the majority of the literature and based on the idea that a window of time exists in which consolidation must occur (Fishbein et al., 1971; Smith et al., 1991), predicted that as the delay between learning and sleep extended, the memory trace would degrade, resulting in a decrease in performance at retest. The opposing

hypothesis, based on the idea that sleep can actively retain or recover memories (Fenn et al., 2003; Backhaus et al., 2007; Baran et al., 2010; Rascmany et al., 2010) predicted that all sleep groups would perform equally, and superior to the wake group, regardless of the length of the imposed delay.

Methods

Participants

Forty-three participants with an average age of 19.75 years (range 18-29) were recruited from the undergraduate population at the City College of the City University of New York. All subjects were reportedly in good health, free of sleep disorders or drugs that might impair or facilitate sleep, as determined by a screening interview. Participants were required to maintain a regular sleep schedule for the week prior to each experimental day, with similar bed and wake times from day to day, as verified by a subjective sleep log. In order to ensure similarity between subjects, they were required to go to bed no later than midnight and awaken no later than 8am. Participants were also asked to refrain from alcohol or unnecessary drugs the day prior to as well as the day of the study, and caffeine the day of the study. Those who failed to meet these requirements were excluded prior to beginning the experiment. Of the original 43 participants, seven subjects were excluded from data analysis due to: inability to fall or remain asleep (3 nap participants), resulting in extended sleep latency and/or excessively fragmented sleep; inability to remain awake (1 wake participant); failure to correctly record responses on the answer sheet (1 participant); or below chance performance at test and retest (2 participants). The remaining 36 participants consisted of 17 males and 19 females. All

participants signed informed consent. This study was approved by the City College of New York Institutional Review Board.

Task

A visual recognition task was utilized in which 150 neutral pictures of non-renowned people, objects, and landscapes, matched for brightness and contrast, were presented to the participants via Microsoft PowerPoint on a 20" computer screen. During the learning phase, participants viewed the pictures in five trials of thirty mutually exclusive pictures, counterbalanced across subjects and separated by two-minute inter-trial intervals. Trials began with a fixation crosshair for 1s, followed by the target picture for 3s, during which subjects simply viewed the stimulus. Each picture was presented in one quadrant of the computer screen, one per slide (**Figure 1.1A**), in order to add a spatial aspect to the task. Each picture slide was followed by a screen prompting the participant to respond via mouse click as to whether the previous picture was an indoor or outdoor scene (**Figure 1.1B**). This enabled confirmation of stimulus viewing, and offered a cogent behavioral marker to confirm that subjects paid attention to the stimuli. This decision was followed by another 1s crosshair fixation before the next picture was presented.

Subjects were not asked to memorize the pictures. Rather, they were given three task objectives prior to the start of the trials. First, they were instructed to "take in" each picture as it was on the screen, noticing what the picture contained. Second, they were to note whether the scene was indoors/outdoors and told they would be asked to respond to this afterward. Finally, they were asked to take notice of which quadrant of the computer

screen the picture appeared. Participants were not informed they would be tested on their memory of this task.

During two testing sessions, 100 new pictures of similar neutral people, objects, and landscapes were intermixed with previously viewed 150 pictures, 1/3 presented during initial baseline testing and 2/3 at retest. As each picture was presented centered on the computer screen (**Figure 1.1C**), participants were required to make an "old/new" decision, recorded on an answer sheet, as well as indicate which corner of the screen the picture had been presented if deemed as "old".

Procedures

At least one week prior to the experimental day, subjects were contacted via email to confirm their intent to participate, informed of prerequisites, and given the sleep log. On the day of the study, participants arrived at the Laboratory for Cognitive Neuroscience and Sleep at 10:00am, signed informed consent, and were introduced to the sound and light attenuated bedroom sleep chambers in order to facilitate adaptation to the surroundings. A brief description of the nature of the experiment was given, questions were answered, and participants completed the first Stanford Sleepiness Scale (SSS).

At 10:30am, nine electrodes were applied to all subjects in preparation for online standard polysomnograph recordings of electroencephalography (EEG; C3-A2, C4-A1), electro-oculography (EOG), and electromyography (EMG) using a five-channel polysomnographic montage in digital EEG acquisition software (Gamma System-Grass/Telefactor^{tm}). In order to reduce experimental confounds, all participants were fitted with electrodes regardless of nap/no-nap grouping, and subjects were not informed

of group assignment until after the learning phase. All subjects' brain activity was monitored online to continuously assess state of sleep/wake.

At 11:00am, subjects were assigned to individual bedrooms for the remaining duration of the experiment. The learning phase then commenced in the bedrooms, with participants seated approximately 2' from the computer monitor. After all 5 trials of pictures had been viewed, participants were immediately tested on a subset of the previously viewed pictures (50 pictures) intermixed with new, similar pictures (35 pictures). They were required to make a check mark on an answer sheet for each picture under either the "new" column or one of 4 "old" columns representing the four quadrants in which the "old" picture could have been presented, thus simultaneously measuring recognition as well as spatial memory. Subjects were allotted as much time as needed to complete this test.

Following this initial testing phase, at approximately 12:00pm, participants were randomly assigned to one of four groups. Of three napping groups, one group immediately took a nap following testing. A second group remained awake until 2:00pm and then napped, while a third group remained awake until 4:00pm and then napped. The final, fourth group remained awake for the entire duration of the experiment (see **Figure 1.2** for Experimental Design). All nap subjects were given a 90-minute sleep opportunity, from the time of lights-out until lights-on. The subject either naturally awakened and remained awake if near the 90-minute mark, or was awakened from stage 1 or 2, as determined using the international criteria of Rechtschaffen and Kales (1968), if the 90-minute mark was near and the subject could potentially progress into a deep stage of sleep before awakening naturally. Subjects were never awakened from SWS or REM

sleep to reduce sleep inertia and the resulting disorientation and confusion experienced when emerging from these stages. While awake, subjects sat in a semi-recumbent position on the bed and passively watched light, animated comedies, chosen to reduce interference with viewed stimuli. They were allowed to eat and drink (non-caffeinated), but remained in the bedrooms aside from restroom breaks within the laboratory.

At 5:45pm, all subjects had electrodes removed and then sat in front of the computer for retesting. As before, participants completed another Stanford Sleepiness Scale and were tested on their recognition of the remaining previously viewed pictures (100 pictures) intermixed with new pictures (65 pictures), marking their answer sheets as described above, taking as much time as needed. Upon completion of this task, the subjects were debriefed on the purpose of the experiment and then allowed to leave.

Results

Performance reflecting recognition and spatial memory was assessed as a within-subject repeated measure immediately after learning and again after the sleep/wake retention period (Test and Retest, respectively). Change in performance between these testing phases was compared between the different conditions (n = 9 per group), groups that napped at intervals (Immediate, 2-Hour, 4-Hour) as well as the control wake group (Wake). Recognition memory was measured as the percentage of correctly identified previously viewed "old" pictures, corrected for false alarm rate, for each test phase. Similarly, spatial memory was measured as the percentage of correctly identified picture locations of previously seen pictures for each test phase.

Sleepiness Measures

The Stanford Sleepiness Scale uses a numerical scale 1-7 (1 being least sleepy, 7 most) to rate levels of alertness/sleepiness. Participants completed two SSS scales, upon arrival to the lab at approximately 10am and again immediately before retest at approximately 6pm. Group means ± SEM for each measure were, respectively; Wake = 1.44 ± .176, 3.44 ± .294; Immediate = 2.00 ± .289, 2.89 ± .351; 2-Hour = 1.67 ± .236, 3.22 ± .324; 4-Hour = 2.22 ± .401, 3.00 ± .333. There were no group differences using these subjective ratings at both the initial testing session (One-way ANOVA, $F_{3,32} = 1.45$, $p = .248$) as well at retest (One-way ANOVA, $F_{3,32} = .570$, $p = .639$).

Sleep Data

One-way ANOVAs were conducted in order to compare sleep data between the three nap groups. No significant differences were found between the conditions for any specific sleep stage or characteristic. All groups had similar total sleep times (mean ± SEM, in min), with the Immediate group averaging 78.11 ± 5.45, 2-Hour 74.94 ± 8.46, and 4-Hour 76.44 ± 6.90 ($F_{2,23} = .051$, $p = .950$). Sleep latency was also similar between groups; Immediate with 9.17 ± 2.42, 2-Hour 6.12 ± 1.45, and 4-Hour 7.2 ± 2.00 ($F_{2,23} = .566$, $p = .576$). When examining the sleep stages, Stage 1 was omitted because it represents a brief transitional stage between wakefulness and sleep, and Stages 3 and 4 were combined in the conventional representation of SWS. Groups did not significantly differ in amount of Stage 2 sleep: Immediate 43.33 ± 5.64, 2-Hour 34.75 ± 4.97, and 4-Hour 38.28 ± 3.20 ($F_{2,23} = .829$, $p = .449$); REM sleep: Immediate 15.22 ± 6.23, 2-Hour 11.94 ± 3.90, and 4-Hour 10.00 ± 3.56 ($F_{2,23} = .316$, $p = .732$); or SWS: Immediate 9.67 ±

3.63, 2-Hour 18.13 ± 4.06, and 4-Hour 20.89 ± 4.06 ($F_{2,23} = 2.32$, $p = .121$). However, when using independent t-tests to examine group differences in amount of SWS, a nearly significant difference was found between the Immediate and 4-Hour groups ($t = -2.07$, $p = .055$). Refer to **Table 1.1** for sleep data synopsis.

Spatial Memory

The percentages of correctly identified spatial locations for previously viewed stimuli were calculated for both the test and the retest sessions (correctly identified locations/total "old" pictures). Average scores for each group during the initial test for each group were as follows (mean %age ± SEM): Wake 32.67 ± 3.00 percent, Immediate 30.78 ± 3.65, 2-Hour 28.89 ± 2.83, 4-Hour 32.36 ± 5.58. During retest, average scores for each group were Wake 19.00 ± 2.37 percent, Immediate 19.33 ± 2.58, 2-Hour 21.22 ± 1.96, and 4-Hour 22.47 ± 4.38. Using repeated-measures ANOVA, where condition (Wake, Immediate, 2-Hour, 4-Hour) served as the between-subject factor, and time of testing (Test/Retest) served as the within-subject factor, a highly significant main effect of time was found ($F_{1,32} = 43.30$, $p < .001$), implying that, in all groups, performance on the spatial task deteriorated over time. No significant interaction between time of test and group was found ($F_{3,32} = 1.15$, $p = .342$).

Recognition Memory

Averaging the percentage of correctly identified "old" pictures, corrected for bias by subtracting the percentage of false alarms for each subject, for the initial test phase (mean %age ± SEM) the control Wake group correctly recognized 76.27 ± 2.52 percent

of the old pictures, 80.29 ± 3.35 for the Immediate group, 80.30 ± 3.73 for the 2-Hour group, and 81.96 ± 2.20 for the 4-Hour group. All participants performed similarly during the initial test session regardless of group, as confirmed by a one-way ANOVA ($F_{3,32} = .644$, $p = .593$). At retest, the Wake group averaged a 59.84 ± 3.68 percent, Immediate 61.53 ± 4.84, 2-Hour 68.87 ± 3.84, and 4-Hour 78.78 ± 3.77 (**Table 1.2**).

Using repeated-measures ANOVA, the change in recognition memory performance from test to retest was examined. Condition (Wake, Immediate, 2-Hour, 4-Hour) served as the between-subject factor, while time of testing (Test/Retest) served as the within-subject factor. There was a highly significant overall main effect of time ($F_{1,32} = 58.31$ $p < .001$), indicating that, in all groups, performance on the recognition task deteriorated over time, from initial test until retest. A significant interaction between time of testing and condition was found ($F_{3,32} = 4.47$, $p = .010$). Post-hoc analyses using Least Significant Difference (LSD) revealed that the 4-Hour delay group performed significantly better, with recognition memory deteriorating less than both the Wake and Immediate groups ($p = .010$, $p = .044$, respectively) (**Figure 1.3**).

Examining the nap groups using Spearman's Rank Correlation revealed a significant negative correlation between the individuals' change in performance from test to retest and the average elapsed time (0, 2, or 4 hours) between initial testing and sleep represented by the nap groups ($r_s = -.623$, $p = .001$). This correlation demonstrates that, while not significantly different from any particular group, the 2-Hour group falls in line with the progression of greater delay before sleep onset equaling better performance (**Figure 1.4**).

Sleep Stages and Time Elapsed before Sleep

While no significant differences between the nap groups in the amount of total sleep time, sleep latency, Stage 2 sleep, REM sleep, or SWS were found, it was noted that the amount of SWS appeared to increase as the length of the delay between learning and sleep extended. Spearman's Rank Correlation revealed a significant positive relationship to confirm this observation ($r_s = .372$, $p = .048$). Due to high levels of variance in the groups on both sleep and performance measures, no other correlations between sleep data and group performance were found.

Discussion

The temporal relationship between learning and memory of a spatial and recognition task was investigated by using a staggered nap design with a waking control to assess the effects of delaying sleep onset. Out of harmony with some studies, a sleep benefit for spatial memory retention was not found. However, after reevaluating the task, it may be reasonable to conclude that the spatial task requirement lacked enough precision to adequately test whether differences exist between sleep and an equivalent period of wakefulness. While it cannot be confirmed in the present study, recalling the visual location of a presented picture may not require the same spatial resources as moving through space, exploring one's environment, as do most animal studies through which knowledge of sleep's contribution to spatial memory processing arise.

On the other hand, it appears that sleep does benefit declarative memory retention compared to an equal amount of time spent awake, although it is clear from the present results that the act of sleeping, alone, is not enough to account for the differences in

retention. In the present experiment, two possible hypotheses regarding the role of sleep in memory retention were entertained, and it was anticipated that the memory trace would either degrade as the length of the delay between learning and sleep increased (consistent with classical interference theory), or that sleep would actively sustain/recover memory retention regardless of the delay, resulting in equal performance among sleep groups, (consistent with systems consolidation theory). However, neither hypothesis was supported. Quite the opposite, better performance on the recognition task was seen the longer participants remained awake before nap onset.

As stated before, subjects were not asked to memorize the pictures and were not informed that they would be tested on their recognition of previously seen pictures. It cannot be ruled out that once the initial test was given, subjects may have anticipated an additional test later in the day. However, this is unlikely to have affected consolidation or subsequent recognition, although recent evidence suggests that expectations of future use of learned material may influence how sleep facilitates consolidation (Wilhelm et al., 2011). Once subjects realized they were to be tested on the material, they had already completed viewing the stimuli, so anticipation of a test did not bias their encoding of the material. The stimuli presented in the two testing sessions were mutually exclusive so that subjects were not re-exposed to any previously viewed stimuli during the first test that would aid them in retest performance. On the off chance that a subject did anticipate a future test and could rehearse the remaining pictures in his/her mind's eye, an attempt to reduce practice effects was made by exposing the subjects to a visual passive activity, watching animation, for the duration of the delay not spent sleeping. While subjective measures were not administered to determine whether or not rehearsal was taking place,

one would anticipate that the occurrence of rehearsal would be evenly spread across the groups and not contribute to the group-specific differences in performance found from test to retest.

The unanticipated finding in the present study lends itself to several possible explanations, which will be considered next.

Neutral vs. Emotional Stimuli Processing during Wake

One possible explanation for the results centers on the task. The declarative visual recognition task consisted of only *neutral* pictures of people, objects, and landscapes. Subjects were asked to view the stimuli with no accompanying narrative or emphasis on the importance of remembering the picture. Reviewing the literature using recognition tasks comparing neutral and emotional stimuli, the majority of studies found benefits to performance after a period of sleep only when the stimuli contained an *emotional* component, making the memory trace stronger and more salient, with corresponding stronger neuronal connections (Hu et al., 2006; Payne et al., 2008; Ritchey et al., 2008; Nishida et al., 2009; Sterpenich et al., 2009). In these studies, employing both emotional and neutral stimuli in a sleep/no sleep design, sleep provided no additional benefit, compared to remaining awake, for performance on neutral items, unlike for emotional items. However, these studies used only one sleep period, usually occurring immediately after learning, in contrast to the delayed sleep paradigm the present study employed. To this extent, the current results are compatible with the literature, with no performance benefit found in the Immediate nap group.

Since increasing the delay between learning and sleep resulted in significantly better performance from learning to retention test, it can be hypothesized that a period of time spent awake, further processing the neutral memories, strengthens the memory trace and makes it more salient, possibly through time-dependent consolidation occurring during a time of passive activities. It is only then, when the memories are strengthened, that sleep can play a role in facilitating the retention of the memory. It is also possible that perhaps distinct aspects of the memory trace are consolidated differentially during wake and sleep, resulting in combined better performance. Such dissociated improvement has been demonstrated, although in procedural memory, providing evidence for different roles for wake and sleep in memory processing (Cohen et al., 2005). While these theories could be plausible, further research, ideally using imaging techniques, would be necessary to confirm reactivation of the memory trace during this resting wake period.

It is also possible that a memory-dependent sleep window for human declarative memory has been uncovered, similar to the paradoxical sleep windows described by Smith (Smith, 1996; Smith et al., 1991). He describes these windows as post-training periods of time in which REM sleep is critical and has been augmented by learning, increasing the amount and length of REM sleep. These critical windows generally occurred several hours, rather than immediately, after rodent mass training and only when sleep occurred in this period did performance benefit. The fact that a period of augmented SWS was found to benefit performance several hours after training is an intriguing parallel with Smith's work, but clearly the evidence to support this claim in humans is weak at this stage.

Circadian Rhythmicity

While use of a daytime nap was intended to diminish the effects of circadian differences between groups in terms of time of learning and testing, held constant in the current study, the delayed nap design did not fully eliminate all possible circadian confounds. In one overnight study, Plihal and Born (1997) explored the differences in declarative and procedural memory processing by different sleep stages by taking advantage of the natural circadian structural differences between SWS and REM sleep, with greater amounts of SWS occurring in the early night and lessening toward morning, while REM sleep increases toward late morning. This homeostatic exchange in amounts of REM and SWS continues during the day, so that a 1pm nap theoretically contains equal amounts of REM sleep and SWS, while a 5pm nap might show more SWS than REM sleep. The present data lends support to this idea. The ratio of SWS to REM sleep during the naps reflects this shift, with the amount of SWS increasing as the delay between learning and sleep increased. At the same time, the amount of REM sleep reciprocally decreased over this time period. This increase in SWS over time is thought to be mediated by increased adenosine release and accumulation over extended waking periods, resulting in greater slow-wave activity at sleep-onset (Radulovacki et al., 1982; Benington et al., 1995; Porkka-Heiskanen et al., 1997; Retey et al., 2005). It should be noted though that homeostatic differences found in this study could only be used to explain performance differences as it specifically applies to the type of sleep each group predominantly achieved, since time of learning and testing was consistent between the groups.

When examining these unusual results in the context of homeostatic changes in sleep over time, one could conclude that the most logical explanation for the performances difference found between the groups may be due to the later nap groups having the lowest level of homeostatic sleep pressure at the time of *retesting*. However, if post-sleep-increased sleep pressure at retest was the cause of these results, then the immediate nap group, whose homeostatic sleep pressure would have been greatly reduced with their noon nap, should be performing far better than the wake group, whose pressure had been building from the time they awakened in the morning until the 6pm retest. One can see, in fact, that this is not the case.

Homeostatic Need for SWS

Greater amounts of SWS seen in the 2-Hour and 4-Hour delay groups compared to the Immediate group may not strictly be due to differences in the time of the nap, but to a neural activity-based increase in the homeostatic need for SWS built up *prior to* the nap. According to Tononi & Cirelli's Synaptic Homeostasis Hypothesis (Tononi & Cirelli, 2003, 2006; Huber et al., 2004) one function of sleep, specifically SWS, is to downscale, or decrease, synaptic weights. restoring synaptic resources and lowering the threshold for firing. Performance on memory tasks for information learned prior to sleep benefits from this downscaling, with weak synaptic connections related to everyday "noise" falling below threshold, while synaptic connections related to learned material, or the "signal", remaining above threshold, increasing the ratio of signal to noise. The more information that is acquired prior to sleep, be it learned information or simple sensory experiences over everyday life, the greater the homeostatic need for SWS and,

consequently, more slow wave activity is seen after sleep onset. Whether the increase in amount of SWS observed is due to this homeostatic increase due to learning alone or in conjunction with circadian rhythmicity, Tononi and Cirelli's theory of the function of SWS lends an elegant mechanistic model for these results.

It must be emphasized that the hypothesized synaptic homeostasis theory is not mutually exclusive from, and may occur in conjunction with, the standard consolidation theory discussed earlier, which gives an active role to slow-wave activity and sharp wave ripples in the reactivation-based shift of new memories from short term to long term stores, resulting in better, more stabilized performance after sleep compared to wake. Given the differences between the groups in amount of SWS attained, this systems consolidation theory can also be used to account for the present unanticipated behavioral data. The Synaptic Homeostasis Hypothesis was the focus in this discussion as it also explains the increases in SWS seen in the groups after learning and subsequent time spent awake as well as lending explanation as to how slow-wave activity benefits performance.

Directions for Future Research

With these unexpected results, new directions for future research present themselves in order to clarify the present findings and possibly provide support to potential mechanistic explanations for the current results. To all of the following possible protocols, a psychomotor vigilance test of general cognitive ability would be useful to aid in differentiating changes in performance due to consolidation effects from possible blanketing changes in ability to complete general cognitive tasks due to potential confounding homeostatic differences between groups.

First, of interest would be a follow-up study comparing performance using both emotional and neutral stimuli, employing the current protocol, in order to examine whether or not the neutrality of the recognition memory task contributed to the findings. Varying the salience of the viewed stimuli, perhaps without the spatial aspect in order to simplify the comparisons, would allow a clearer picture of the benefit of sleep for different types of recognition memory.

Another possible area of interest is the extent of the delay time between learning and sleep onset. In order to more clearly map the time-dependent benefit of sleep on memory, the delay could potentially be extended over a greater period of time, while still holding the time of learning and testing constant, with more napping groups introduced in the interim.

Finally, focusing on SWS as discussed at length both in regard to sleep-dependent systems consolidation theory as well as the Synaptic Homeostasis Theory, a follow-up study in which amount of SWS is somehow held constant between all nap groups over the delay may aid in understanding the contribution of this type of sleep to declarative recognition memory processing. While it cannot be said with certainty, the possibility exists that had all nap groups achieved equal amounts of SWS, support for the hypothesis that sleep actively retains the memory trace may have been discovered, with equal performance across nap groups, regardless of the length of the delay. One method of possibly controlling for amount of SWS as well as homeostatic confounds would be to hold constant the time at which all groups napped and were subsequently retested, but stagger the pre-nap times of training and initial testing, so that, similar to this study, groups would learn 4 hrs, 2 hrs, or immediately prior to napping. This would eliminate

homeostatic sleep differences, although groups would be learning at different times of the day, potentially confounding in itself. There are inherent confounds with manipulating the natural amount of SWS achieved in a nap as well, either through truncating the length of the nap or inducing slow waves to boost slow wave activity in the earlier nap groups, but the idea of exploring this area in future projects if proper controls can be achieved is appealing and would certainly add to our understanding of the role for SWS in memory processing.

Study II: The effect of short and long daytime naps on bimodal associative learning

Introduction

The idea that sleep contributes to the processing of new memories is widely accepted. Behavioral and physiological studies of both animals and humans using declarative and procedural tasks have repeatedly demonstrated better retention of the learned material after a period of sleep, compared to an equal period spent awake. However, the questions of how much sleep and of what type of sleep are necessary to see lasting benefits remain. Daytime naps of 60-90 minutes have been found to benefit memory similarly to overnight sleep, providing an economic method to both study sleep and obtain memory facilitation (Mednick et al., 2003; Schabus et al., 2005; Backhaus & Junghanns, 2006; Tucker et al., 2006; Lau et al., 2010). A nap of this length typically contains Stage 1 and 2 sleep, SWS, and potentially a period of REM sleep as well. What is unknown is whether a nap of a shorter duration, not containing SWS or REM sleep, generally thought to be the predominant stages facilitating memory, will also result in better memory compared to remaining awake.

There is an ongoing debate as to whether sleep plays an active or a passive role in memory processing, or perhaps something in-between, a permissive role (Ellenbogen et al., 2006). Both the passive and permissive theories assume that there is nothing inherent in the state of sleep that aids memory processing. Rather, it is the reduction of interference during sleep that allows information to consolidate more efficiently than while awake. Conversely, the active hypothesis gives the state of sleep, specifically SWS when discussing declarative memory, a unique role in facilitating the stabilization and

strengthening of a memory trace that cannot be achieved in other states. The emphasis of the experimental design implemented in this study addresses the length of nap issue, but, moreover, attempts to tackle the passive/persistent/active controversy.

A 2008 paper by Lahl et al. described the results of two experiments using free recall testing of previously viewed word lists after a retention period containing wakefulness, a short nap, or a long nap. They found that an ultra short, 6-minute nap benefited memory, although not as large a benefit as a longer nap provided. However, within the nap groups, they found no correlation between performance and total sleep time (TST) or other sleep parameter. They, therefore, concluded that the onset of sleep potentially initiates a cascade of consolidation continuing even after awakening a short time later. While the results of this study are intriguing, there are a few questions related to the consequences of consolidation that are not addressed. The process of consolidation is thought to strengthen a new memory trace as well as stabilize and protect it from subsequent interference. While the Lahl et al. study examined short-term retention after sleep, they did not explore further than this, details that the present design aimed to address.

In the present study, subjects who obtained a brief 10-min nap, containing only Stages 1 and 2 sleep, were compared to those who either remained awake or achieved a 60-min nap, containing SWS, in order to attempt to replicate the short-term benefit of sleep on declarative memory found in the Lahl et al. study. Additionally, the present study implemented a protocol adapted from one proposed by Ellenbogen et al. (2009) to address the passive/permissive/active conundrum and investigate whether a brief nap protects a memory trace from a subsequent stimulus-related interference task, as well as

examined long-term consolidation persisting for a one-week retention period. Using a unique bimodal paired-associates task, which pairs visually presented words with recognizable sounds to create a complex memory, it was hypothesized that SWS must be achieved in order to actively protect and consolidate declarative memory, rather than simply the act of falling asleep alone.

Methods

Participants

Forty-one participants with an average age of 19.54 years (range 18 to 28) were recruited from the undergraduate population at the City College of the City University of New York via an online voluntary subject pool through which students make appointments to participate in projects of their choosing. All subjects were reportedly in good health, free of sleep disorders or drugs that might impair or facilitate sleep, as determined by a screening interview. Participants were required to maintain a regular sleep schedule for the week prior to each experimental day, with similar bed and wake times from day to day, as verified by a subjective sleep log. In order to ensure similarity between subjects, they were required to go to bed no later than midnight and awaken no later than 8am. Participants were also asked to refrain from alcohol or unnecessary drugs the day prior to as well as the day of the study, and caffeine the day of the study. Those who failed to meet these requirements were excluded prior to beginning the experiment. Of the original 41 participants, five subjects were excluded from data analysis due to: inability to fall or remain asleep (1 nap participants), resulting in extended sleep latency and/or excessively fragmented sleep; inability to remain awake (1 wake participant);

statistically outlying poor performance during testing (1 participant); and performance reflecting a ceiling effect (2 participants). The remaining 36 participants consisted of 8 males and 28 females, reflecting the uneven distribution of sexes enrolled in psychology courses at the university. All participants signed informed consent. This study was approved by the City College of New York Institutional Review Board.

Tasks

Bimodal Paired-Associates Task

The bimodal paired-associates task was created as a modified version of the traditional verbal paired-associates task used in many declarative learning studies. The task consisted of 36 two-syllable nouns ranked high (>6 on 7 pt. scale) for both concreteness and imagery, chosen from the Toronto Word List (Friendly et al., 1982) and randomly paired with 36 2-second sound clips of highly concrete, easily recognizable sounds. Sounds were chosen by compiling duplicate categorical suggestions from 50 polled individuals, arriving at 6 categories (animal, vehicle, human sounds, machines/appliances, sounds in the home, nature) with 6 easily recognizable sound clips per category. All sound clips were controlled and matched for volume level, duration, and complexity. Participants were seated in front of a desk mounted 20" computer monitor approximately 2 feet from the screen. During the learning phase, participants heard/viewed 36 audio/visual (A/V) stimuli via a Microsoft PowerPoint presentation. Participants were instructed to pay close attention to each pair and try to associate the sound in the audio clip and the word displayed on the screen together using whatever strategy worked for them. For each pair, the audio clip was presented first, for 2 seconds,

immediately followed by the paired word, presented in the middle of the screen for 2 seconds. The A/V pair was then presented simultaneously for two seconds, followed by an interstimulus delay of 3 seconds with a crosshair fixation point mid-screen. The entire 36 pairs were presented once through. Immediately following the presentation, all 36 pairs were tested for encoding using randomized cued recall, in which the audio clips were presented alone and subjects asked to write the paired visual words into the provided space on an answer sheet. Responses were immediately scored for accuracy and followed by another run-through of the 36 pairs until participants performed better than a 75% criterion level. A criterion level of 75% was set to ensure that subjects learned the material well, but still had room for possible sleep facilitated improvement. Hippocampal activation during sleep has been found to be contingent on acquisition level during training (Peigneux et al., 2003; Tucker & Fishbein, 2008). No feedback was given during the encoding tests.

Participants were tested three times on three counterbalanced, mutually exclusive subgroups of 30 of the learned A/V pairs (10 pairs per test session), with the first and last 3 pairs removed from the pool to control for primacy and recency effects.

Interference Task

This task was inspired by a task designed by Ellenbogen et al. (2009), modified to reflect the bimodal stimuli used in this study. Participants were trained on a stimulus-related interference task immediately following the first testing session, to >90% criterion level to ensure task encoding. A higher criterion level was used for the interference task because it was not necessary to allow for improvement. This task involved learning new

audio/visual pairs corresponding with 10 of the untested (A_2/V_2) pairs to create 10 interfering pairs (A_2/V_{Int}) with new words paired with previously learned sounds. Learning occurred in the same manner as the original pairs.

Procedures

At least one week prior to the experimental day, subjects were contacted via email to confirm their intent to participate, informed of prerequisites, and given the first sleep log. On the day of the study, participants arrived at the Laboratory for Cognitive Neuroscience and Sleep at 11:00am, signed informed consent, and were introduced to the sound and light attenuated bedroom sleep chambers in order to facilitate adaptation to the surroundings. A brief description of the nature of the experiment was given, questions were answered, the week's sleep logs were collected, and the following week's sleep logs were distributed.

At 11:30am, nine electrodes were applied to all subjects in preparation for online standard polysomnograph recordings of electroencephalography (EEG; C3-A2, C4-A1), electro-oculography (EOG), and electromyography (EMG) using a five-channel polysomnographic montage in digital EEG acquisition software (Gamma System-Grass/Telefactor^{tm}). In order to reduce experimental confounds, all participants were fitted with electrodes regardless of nap/no-nap grouping, and subjects were not informed of group assignment until after the learning phase. All subjects' brain activity was monitored online to continuously assess state of sleep/wake.

At 12:00pm each participant was directed to a bedroom for the remainder of the experimental day. They completed the first of 3 Stanford Sleepiness Scales (SSS), then

sat before a computer monitor and spent approximately 45 minutes learning the 36 audio/visual (A/V) pairs to criterion. Immediately after the training session (at approximately 1:00pm), participants were randomly assigned to either lie down for a nap or remain awake engaged in a passive activity (i.e. watching a nature video). Subjects were divided into one of three groups: a wake group, a 10-minute nap group, or a 60-minute nap group (**Figure 2.1**). The 10-min nap group was awakened after approximately 10 minutes of sleep, with timing beginning with the first two consecutive epochs of Stage 1 sleep. The 60-minute nap group was given a 60-minute sleep opportunity, from the time of lights-out until the subject either naturally awakened or was woken from stage 1 or 2, as determined using the international criteria of Rechtschaffen and Kales (1968). Subjects were not woken from SWS or REM sleep to reduce sleep inertia and the associated disorientation and confusion. After the nap period, subjects remained awake and engaged in a passive activity. At 2:30pm, participants completed the second SSS and were then tested on the first 10 of the learned audio/visual (A_1/V_1) pairs, again using cued-recall in which the sound was played and participants recorded the word on an answer sheet. This test measured short-term memory of the learned material.

Immediately following this testing period, subjects were trained on the interference task (A_2/V_{Int}). After a 10-minute delay, during which subjects sat quietly with no visual or auditory stimulation, participants were tested using cued recall. The sound clip (A_2) was presented as the cue and the participants were asked to recall both the original learned paired word (V_2) and the new interfering paired word (V_{int}) to avoid competition among responses, although the recalled item of interest was the original

paired word, V_2. This test was used to measure the stabilization and protection of the memory trace. After completion of this test session, participants had their electrodes removed and were allowed to leave the laboratory.

One week after initial participation, participants returned to the laboratory at 11:30am and completed the final SSS. They were then tested on the remaining 10 originally learned audio/visual pairs (A_3,V_3). This test provided a measure as to how long-lasting the potential benefit of sleep was. Subjects were then debriefed, credited, and thanked for participation.

Results

Performance on the bimodal paired-associates task was assessed after a nap/no-nap interval (Test 1), after a stimulus-related interference task (Test 2), and again after a week of retention (Test 3). These measures were taken to assess short-term stabilization of learned material, protection of a memory from interference, and long-term consolidation after sleep versus no sleep, respectively. Subjects were trained on the paired-associates task to a minimum 75% criterion level to ensure subjects (n = 12 per group) encoded the material similarly, before being divided into one of three conditions (Wake, 60-min Nap, 10-min Nap). Performance was calculated by dividing the correctly recalled words, which had been paired to the cued sound clips during learning, by the total number of possible correct answers to arrive at a percentage correct score for each test session (see **Table 2.1** for a summary). Answers that were incorrect, yet semantically related or visually similar to the target word (e.g. castle vs. palace, figure vs. finger), were given half credit.

Sleepiness Measures

The Stanford Sleepiness Scale uses a numerical scale 1-7 (1 being least sleepy, 7 most) to rate levels of alertness/sleepiness. Participants completed three SSS scales, upon arriving at the laboratory immediately before beginning the learning session at approximately 12:00pm, before the first test session occurring after the nap/no-nap interval at approximately 2:30pm, and again one week later before the final test session at approximately 11:30am. Group means ± SEM for each measure were, respectively: Wake = 2.75 ± .131, 3.25 ± .179, 1.75 ± .179; 10-min Nap = 3.33 ± .284, 3.33 ± .432, 2.08 ± .229; 60-min Nap = 2.83 ± .271, 2.67 ± .333, 1.92 ± .313. There were no group differences using these subjective ratings using One-way ANOVA at any session (SSS1 $F_{2,33} = 1.75$, $p = .190$; SSS2 $F_{2,33} = 1.20$, $p = .314$; SSS3 $F_{2,33} = .457$, $p = .637$).

Bimodal Paired-Associates Memory

All subjects were trained to a minimum criterion level of 75% while those who scored higher than 95% during learning were excluded to avoid a ceiling effect. Mean percentage scores during the learning session for each group ± SEM were as follows: Wake = 77.58 ± 1.58, 10-min Nap = 80.75 ± 1.57, 60-min Nap = 81.33 ± 2.29. A One-way ANOVA revealed that the groups encoded the information similarly ($F_{2,33} = 1.06$, $p = .358$). The number of trials necessary to reach criterion were also calculated for each group and the mean ± SEM for each group was: Wake = 2.50 ± .261, 10-min Nap = 2.67 ± .225, 60-min Nap = 2.08 ± .193. A One-Way ANOVA showed that the groups were not significantly different on this as well ($F_{2,33} = 1.74$, $p = .192$).

Using a One-way ANOVA to analyze performance at Test 1, sleep's impact on the short-term stabilization of the new memory trace was examined. Mean recall percentage ± SEM for each group was: Wake = 75.83 ± 3.58, 10-min Nap = 84.17 ± 2.88, 60-min Nap = 90.00 ± 2.13. A significant difference was found ($F_{2,33} = 5.93$, $p = .006$) between the groups. Post hoc analysis using Least Significant Difference (LSD) showed that the Wake group performed significantly worse than the 60-min Nap group ($p = .002$) and trended toward significantly worse performance compared to the 10-min Nap group ($p = .052$).

In order to measure the effects of sleep on the protection of a new memory trace from competing interference, change in performance from Test 1 to Test 2 was examined. The mean percentage scores ± SEM on Test 2 A_2/V_2 recall were calculated, which measured recall of words originally paired with cued sounds after interference (Wake = 57.08 ± 3.51, 10-min Nap = 60.42 ± .234, 60-min Nap = 75.83 ± 2.03). Using repeated-measures ANOVA, with Condition (Wake, 60-min Nap, 10-min Nap) as the between-subject factor and Interference (No Interference=Test 1, Interference=Test 2 A_2/V_2) as the within-subject factor, a highly significant main effect of Interference was found ($F_{1,33} = 176.31$, $p < .001$) indicating that, in all groups, the related interference task compromised the paired-associates memory trace. A significant interaction between Interference and Condition was found ($F_{2,33} = 3.78$, $p = .033$), and post hoc analyses using LSD revealed that the 60-min Nap group performed significantly better, with interference disrupting the memory trace far less than both the Wake and 10-min Nap groups ($p < .001$, $p = .006$, respectively)(**Figure 2.2**). All subjects learned the interfering pairs to similar percentages (Wake = 93.33 ± 2.84, 10-min Nap = 95.42 ± 1.68, 60-min Nap = 96.67 ±

1.42; One-way ANOVA $F_{2,33} =$ .658, p = .524). However, analysis revealed that the Wake group needed significantly more trials to learn the material to the same level (Wake = 2.67 ± .188, 10-min Nap = 2.08 ± .083, 60-min Nap = 1.75 ± 179; One-Way ANOVA $F_{2,33} = 8.67$, p = .001).

In order to measure the effects of sleep on long-term consolidation of the paired-associates, the change in performance from Test 1 to Test 3 was examined. Test 3 performance, mean recall percentage ± SEM, for each group was: Wake = 37.50 ± 6.85, 10-min Nap = 37.50 ± 3.05, 60-min Nap = 60.00 ± 2.75. Using repeated measures ANOVA with Condition (Wake, 10-min Nap, 60-min Nap) as the between-subject factor and Time (Test 1, Test 3) as the within-subject factor, a highly significant main effect of Time was revealed ($F_{1,33} = 239.68$, $p < .001$). This finding indicates that all groups' performance deteriorated over the week-long retention period regardless of the subjects having slept or remained awake after learning. A significant interaction between Condition and Time was found ($F_{2,33} = 3.78$, p = .033), and post hoc analysis using LSD revealed that the 60-min Nap group retained the learned material from Test 1 to Test 3 better than both the Wake and 10-min Nap groups ($p < .001$, p = .004, respectively)(**Figure 2.3**).

Sleep Data

PSG recordings were scored for the 10-min and 60-min nap groups according to Rechtschaffen and Kales standard scoring criteria. Total sleep time (TST), sleep latency, Stages 2, 3, and 4, slow wave sleep (stages 3+4), and REM sleep were calculated for each condition. Mean ± SEM in minutes for the 10-min Nap group for each parameter was:

TST = 10.75 ± .305, latency = 6.21 ± .667, S2 = 8.21 ± .570, and 0 minutes for remaining parameters. Mean ± SEM in minutes for the 60-min Nap group for each parameter was: TST = 52.50 ± 2.66, latency = 7.67 ± 1.33, S2 = 28.08 ± 2.37, S3 = 10.38 ± 1.70, S4 = 3.25 ± 1.48, SWS = 13.63 ± 2.47, REM = 4.00 ± 1.54 (see **Table 2.2** for a summary). While the two sleep groups were not comparable on all measures, an independent t-test comparing the sleep latencies of the two groups found them to be similar ($t = .979$, $p = .338$).

Correlation and regression analyses were conducted to determine if any particular stage of sleep could predict or explain performance in the two sleep groups. However, no significant statistics were found for any parameter after analyses.

Discussion

The present study investigated the stabilizing and protecting effects of a period of sleep containing SWS, compared to a shorter nap reaching only Stage 2 or a period spent awake, on newly encoded bimodal pairs of sounds and written words. Similar to results found by Lahl et al. (2008), who used an ultra-short 6-min nap, a short-term benefit to memory in both nap groups was revealed, although not as great in the 10-min nap group as the 60-min nap group. In their study, Lahl et al. attributed the retention of memory within the short nap group, which was greater than the waking group but not as strong as a longer nap group, to a possible cascade of consolidating processes begun merely with the onset of sleep. While this possible explanation gives an active role to sleep, the results could very well fit with the passive theory of the role of sleep in memory processing, in that it was simply the time spent "off-line", without the ability for outside

interference to disrupt the memory trace, which allowed better retention. It also fits within the permissive theory framework, that the off-line period prevented interference and also benefited retention as it occurred within a critical window of time.

In order to clarify these results and delve further into the passive/permissive/active issue, two additional components were added to the present study. The first of which was to incorporate the stimulus-related interference task. As suggested by Ellenbogen et al. (2009), introducing this particular interference task, which required subjects to learn to pair competing words with previously paired sounds and then test them on their recall of both the old and newly paired words, tapped into the stabilizing and protective aspect of active consolidation processes. Subjects were tested first on their pure recall of the learned material after the sleep/no sleep retention interval (Test 1) and then immediately trained and tested on the interference task (Test 2). If sleep simply serves in a passive or permissive capacity, allowing consolidation of the learned material to occur more efficiently than it would while awake due to relative lack of interference, then all the subjects who slept should either perform the same on the interference test as they did on the first recall test, all protected from subsequent interference, or at least be similarly impaired. However, it was found that, while all groups were impaired by the interference task, the 60-min nap group's memory was relatively preserved compared to the 10-min nap group, whose performance declined to nearly as poor as the wake group. This finding suggests that the short-term benefit found in the 10-min nap group after the sleep/wake retention was just that, short-term and not reflective of true consolidation.

The second component added to this study not present in the Lahl et al. study was an exploration of the persistence of the memory trace over time. Subjects returned to the lab after a week-long retention period and those who had obtained a 60-min nap retained significantly more learned bimodal pairs than the 10-min nap and wake groups, who performed virtually the same as one another. The benefits seen in the 10-min nap group after the initial sleep/no sleep interval were nonexistent after a week, indicating that simply spending a period of time "off-line" is not sufficient for long-term consolidation of the learned material. Again, if sleep served in a passive or permissive role, rather than actively contributing to the processing of the new information, then benefits proportional to the amount of time spent sleeping should persist over time in both nap groups.

The results of the present study conclusively lend support to an active role for sleep in the consolidation of declarative memory. The subjects in the 60-min nap group all obtained a period of SWS, the prime stage thought to be implicated in hippocampal-dependent memory processing. Both the standard consolidation theory and the homeostasis theory can be used to explain these results. In those who obtained SWS, the memory trace had actively begun the consolidation process and/or the potentiated synapses had been downscaled, increasing the signal to noise ratio and allowing the memory to stand out, an advantage that persisted for at least a week compared to those who did not reach SWS. Although the memory degraded in all groups over time, it was significantly more preserved after having been actively processed by SWS. While the interference task impaired memory for all subjects, there was a marked difference in the impairment of those who had actively processed the information versus those who briefly slept or remained awake. Standard consolidation theory would attribute this advantage to

the strengthening of the synaptic connections of the memory representation through reactivation and the "transfer" from short-term to long-term stores, protecting the memory from new, competing information. When considering the homeostasis theory, SWS served to replenish and reset the synaptic properties, allowing new information to be encoded, and perhaps protecting the original memory by "freeing up" synaptic real estate and avoiding competition of synaptic resources.

Interestingly, the participants who remained awake required significantly more trials to learn the interference task to criterion, compared to both sleep groups. Because subjects reported similar levels of sleepiness on the SSS, this difference cannot be attributed to fatigue or lack of interest. It may be the consequence of higher synaptic thresholds in the Wake group compared to both sleep groups, but significantly different from the 60-min nap group, who experienced synaptic downscaling during SWS, allowing for greater encoding efficiency.

While the present study was able to replicate, in the 10-min nap group, similar benefits found with an ultra short 6-min nap, it is apparent that this is not due to active processing of the information by sleep. It remains unclear as to what caused this temporary preservation of the memory for the paired-associates. While the self-reported sleepiness scales reflect that all groups were similarly alert, it may be that these measures did not pick up on other differences in mood that may have the potential to confound the results, although this is unlikely. Perhaps with a short nap, subjects felt more relaxed and were better able to focus on the task occurring shortly after awakening. While it may have been beneficial to perhaps use a vigilance task or other subjective reporting, it does not diminish the findings regarding the long-term and protective aspects of attaining SWS

sleep. Had the 10-min nap subjects been performing better due to a more relaxed, focused state, this should have carried over to the interference task, which followed immediately after this initial test. It is more likely that there is some truth to passive benefits of sleep, in that the lack of interference during the initial retention period allowed the subjects to better hold the memory in short –term stores for recall, but did little to store and stabilize the memory beyond this.

An interesting correlation anticipated but not found was one between performance and amount of SWS or TST within the 60-min nap group. One could hypothesize that as more SWS was achieved, subjects would perform better during the testing sessions. However, previous studies, including Lahl et al. (Tucker et al., 2006), also failed to find a significant correlation between performance and TST or amount of SWS within sleep conditions. When reflecting on the results from Study I, it was found that the longer the subjects were awake (explored up to 4 hours post-learning), the better they performed from test to retest on a recognition memory test. Additionally, the longer the subjects were awake before napping, more SWS was seen in the nap. The conclusion, based on the mechanistic theories involving a role for SWS in declarative information processing, was that the significantly great amount of SWS present in the later nap group was possibly responsible for better performance. However, in that study, due to the high variability between subjects for both amount of SWS and change in performance, no significant correlation was found between these variables. It is possible that this also explains the inability to find a correlation between SWS and performance in the present study. No correlation was found between Stage 2 sleep and performance, and only 5 of 12 subjects in the 60-min nap group entered REM sleep, with no correlation found in that

subgroup as well. It is inconclusive whether the amount of SWS achieved in a nap is a factor deciding how well a person performs on a declarative task, or whether the act of entering the state of SWS begins consolidation or downscaling of the synapses that carries through beyond the end of the stage. Either way, it is clear from the results of this study that SWS is the key state in which the brain is able to actively facilitate the declarative memory.

Study III: The effects of a daytime nap on subsequent bimodal associative learning

Introduction

The literature examining the benefits of a period of sleep following a learning experience far outweighs that exploring whether sleeping *prior* to learning prepares the brain to encode, store, and retrieve new information. Within the published studies investigating this research question, the experimental subjects, recording modalities, methods, and experimental parameters are so vastly different that there is little consensus as to the role of sleep. Several early animal studies using partial sleep deprivation, selectively eliminating paradoxical, or REM, sleep prior to the acquisition of a learned behavior, demonstrated a resulting impairment to subsequent long-term consolidation, despite both deprived and normally sleeping animals having acquired the behavior equally (Fishbein, 1970, 1972; Hartmann & Stern, 1972, Sagales & Domino, 1973; Silva et al., 2004), although other studies failed to demonstrate this same impairment. More recent work implementing one night of total sleep deprivation in humans resulted in a significant impairment in the ability to encode new episodic information, displaying reduced hippocampal activation, necessary for declarative memory formation, during encoding in the deprived subjects (Yoo et al., 2007). While these studies support the need for sleep for maximal encoding and consolidation, they are, nonetheless, deprivation experiments, which tend to speak more to the negative effects of lack of sleep, such as fatigue, rather than the benefits of normal processing during sleep.

In humans, many early studies concluded that a period of sleep prior to learning had a *detrimental* effect on learning ability. For example, Worchel & Marks (1951) had

subjects learn to pronounce a list of nonsense syllables to a criterion level after either sleeping for 1.5 hours (from 10:00-11:30pm) or remaining awake, and found that when they slept prior to learning, they required more trials to learn and made more errors in pronunciation during learning. They attribute their findings as possibly due to impairing physiological processing occurring during that 1.5 hours of sleep, or, alternatively, to having been in a "warmed up" state of mental preparedness during the waking condition. Several other early studies demonstrated this disadvantageous effect of prior sleep on learning (Stones, 1973; Ekstrand et al., 1977; Grosvenor & Lack, 1984; Tilley & Statham, 1989), using various tasks, citing sleep's impairment of encoding, retention, retrieval, and/or future consolidation. The possible explanations for these findings most often included lower physiological arousal at time of learning and/or the presence of a potential biochemical substance, such as a growth hormone (Ekstrand, 1977), or an impairing physiological process initiated during sleep that carried over into waking and disrupted normal consolidation. These studies all conducted the learning sessions after a period of nocturnal sleep or delayed waking state, meaning that subjects either briefly slept during their normal sleep time and were awakened to learn shortly thereafter, or remained awake past their normal bedtime in the waking condition. Neither of these conditions are ideal when taking into account peaking homeostatic sleep pressure and/or sleep inertia when keeping subjects up later than their bedtime or waking them out of early night sleep, which is deep-sleep rich. The inherent confounds arising from such an arrangement may disguise the true nature of prior sleep's influence on learning.

More recent studies have taken into account the confounding effects of sleep deprivation and the interruption of nocturnal sleep, while still examining the role of sleep

prior to learning, by using a nap protocol. Nishida et al. (2009) and Axmacher et al. (2009), both designed experiments in which subjects were shown sets of pictures both prior to napping, which they called remote memory, as well as after the nap, which they called recent memory, and tested on their recognition of previously viewed pictures. In Nishida et al.'s study, the stimuli were both neutral and emotional, while Axmacher et al. only used neutral stimuli. However, perhaps due to different experimental parameters, they came to conflicting conclusions, with Axmacher demonstrating prior sleep's benefit to recent neutral memory while Nishida found a post-learning sleep benefit only to remote emotional memory, further muddling our understanding of sleep's role pre-learning.

The present study focuses on declarative associative memory, shown to be robustly hippocampal dependent and able to benefit from a period of subsequent sleep compared to wake, specifically SWS, as demonstrated in previously published studies as well as in Study II. Van Der Werf et al. (2009) demonstrated the need for pre-learning SWS when they achieved acoustic suppression of slow wave activity, without loss of sleep or detrimental deprivation effects, and discovered subsequent encoding impairment of declarative material. They observed decreased hippocampal activation during learning after a night of reduced slow wave activity, which, in turn, affected the acquisition of new information into the hippocampus. When examining these results in the framework of either the standard consolidation theory or the Synaptic Homeostasis Hypothesis (Huber, 2007), pre-learning SWS can be thought of as a mechanism that allows the "freeing up" of synapses involved in a memory trace, either through transfer of reactivation dependence to the neocortex (consolidation) or through synaptic downscaling

(homeostasis hypothesis), allowing subsequent new information to be encoded and more efficiently processed. A recent study by Mander et al. (2011) had subjects learning episodic material at 12:00pm and again at 6:00pm, either with or without an intervening nap. In the no-nap condition, ability to learn declarative information degraded over the waking interval. Conversely, the nap condition saw their learning ability restored by the period of sleep, with a positive correlation found between learning ability after the nap and Stage 2 sleep and fast sleep spindles. Sleep spindles, found to be temporally correlated with hippocampal sharp-wave ripples (Siapas & Wilson, 1998; Sirota et al., 2003), are thought to reflect declarative memory processing consistent with the standard consolidation theory, which then, in turn may lead to restored encoding ability for future information.

In an attempt to clarify the diverse, conflicting findings, the present study used a straightforward design in which subjects either achieved a 60-min nap or remained awake, and were then trained to criterion on a bimodal paired-associates task, a variation of a commonly used declarative task. Subjects were compared on their encoding ability, as measured by the number of trials required to learn to criterion as well as a baseline test on a portion of the learned material, short-term retention following an hour retention interval of wakefulness, and long-term consolidation, as tested 24 hours after training. The nap protocol was used to control for deprivation effects, adding beneficial sleep to a normal waking state rather than examining detrimental effects of sleep loss. Sleepiness measures were included to address potential arousal differences between napping and wake groups. It is hypothesized that a daytime nap will result in greater encoding

efficiency, as measured by the number of trials needed to reach criterion, and superior short-term and long-term memory, as compared to remaining awake.

Methods

Participants

Twenty-eight participants with an average age of 20.76 years (range 18 to 31) were recruited from the undergraduate population at the City College of the City University of New York. All subjects were reportedly in good health, free of sleep disorders or drugs that might impair or facilitate sleep, as determined by a screening interview. Participants were required to maintain a regular sleep schedule for the week prior to each experimental day, with similar bed and wake times from day to day, as verified by a subjective sleep log. In order to ensure similarity between subjects, subjects were required to go to bed no later than midnight and awaken no later than 8am. Participants were also asked to refrain from alcohol or unnecessary drugs the day prior to as well as the day of the study, and caffeine the day of the study. Those who failed to meet these requirements were excluded prior to beginning the experiment. Of the original 28 participants, three subjects were excluded from data analysis due to: inability to remain awake (1 participant); statistically outlying poor performance during testing (1 participant); and performance reflecting a ceiling effect (1 participant). The remaining 25 participants consisted of 8 males and 17 females, reflecting the uneven distribution of students enrolled in psychology courses at the university. All participants signed informed consent. This study was approved by the City College of New York Institutional Review Board.

Bimodal Paired-Associates Task

The bimodal paired-associates task was created as a modified version of the traditional verbal paired-associates task used in many declarative learning studies. The task consisted of 66 two-syllable nouns ranked high (>6 on 7 pt. scale) for both concreteness and imagery, chosen from the Toronto Word List (1982) and randomly paired with 66 2-second sound clips of highly concrete, easily imaginable sounds. Sounds were chosen by compiling duplicate categorical suggestions from 50 polled individuals, arriving at 6 categories (animal, vehicle, human sounds, machines/appliances, sounds in the home, nature) with 11 easily recognizable sound clips per category. All sound clips were controlled and matched for volume level, duration, and complexity, with volume being no louder than that of human speech. Participants were seated in front of a desk mounted 20" computer monitor approximately 2 feet from the screen. During the learning phase, participants heard/viewed 66 audio/visual stimuli via a Microsoft PowerPoint presentation. Participants were instructed to pay close attention to each pair and try to associate the sound in the audio clip and the word displayed on the screen together using whatever strategy worked for them. For each pair, the audio clip was presented first, for 2 seconds, immediately followed by the paired word, presented in the middle of the screen for 2 seconds. The audio/visual pair was then presented simultaneously for two seconds, followed by an interstimulus delay of 3 seconds with a crosshair fixation point mid-screen. The entire 66 pairs were presented once through. Immediately following the presentation, all 66 pairs were tested for encoding using randomized cued recall, in which the audio clips were presented alone

and subjects asked to write the paired visual words into the provided space on an answer sheet. Responses were immediately scored for accuracy and followed by another run-through of the 66 pairs until participants performed better than a 75% criterion level. A criterion level of 75% was set to ensure that subjects learned the material well, but still had room for possible sleep facilitated improvement. Hippocampal activation during sleep has been found to be contingent of acquisition level during training (Peigneux et al., 2003; Tucker & Fishbein, 2008).

Participants were tested three times on three counterbalanced, mutually exclusive subgroups of 60 of the learned A/V pairs (20 pairs per test session), with the first and last 3 pairs removed from the pool to control for primacy and recency effects. Participants were tested after a 10-minute delay post-learning on the first 20 of the learned audio/visual (A_1/V_1) pairs, using cued recall. After an hour of passive activity, participants were tested on another set of previously learned 20 (A_2/V_2) pairs. One day after initial learning, participants were tested on the final 20 pairs. (A_3/V_3).

Procedures

At least one week prior to the experimental day, subjects were contacted via email to confirm their intent to participate, informed of prerequisites, and given the first sleep log. On the day of the study, participants arrived at the Laboratory for Cognitive Neuroscience and Sleep at 11:45am, signed informed consent, and were introduced to the sound and light attenuated bedroom sleep chambers in order to facilitate adaptation to the surroundings. A brief description of the nature of the experiment was given, questions were answered, and sleep logs were collected.

At 12:00pm, nine electrodes were applied to all subjects in preparation for online standard polysomnograph recordings of electroencephalography (EEG; C3-A2, C4-A1), electro-oculography (EOG), and electromyography (EMG) using a five-channel polysomnographic montage in digital EEG acquisition software (Gamma System-Grass/Telefactor^{tm}). In order to reduce experimental confounds, all participants were fitted with electrodes regardless of nap/no-nap grouping, and subjects were not informed of group assignment until after the learning phase. All subjects' brain activity was monitored online to continuously assess state of sleep/wake.

At 12:30pm, participants were randomly divided into one of two groups, a wake group or a 60-minute nap group, and directed to assigned bedrooms to either lie down for a nap or remain awake engaged in a passive activity (i.e. watching a short nature video). After the nap period, participants were allowed to fully return to a waking state before training began.

At 1:45pm, participants completed the first of three Stanford Sleepiness Scales (SSS). They then sat before a computer monitor in the bedroom and spent approximately 30 minutes learning the audio/visual (A/V) paired associates to criterion. Following learning and a subsequent 10-minute delay, during which subjects sat quietly without visual or auditory stimulation, subjects were tested on their recall of the first 20 of the learned audio/visual (A_1/V_1) pairs, using cued recall. This test served as a baseline measure to ensure both groups learned the material similarly. After this initial testing session, all participants were engaged in passive behavior (i.e. watching another nature video) for one hour. At approximately 3:45pm participants completed the second SSS and were tested on their recall of a second set of previously learned 20 (A_2/V_2) pairs in

order to measure pre-learning sleep's contribution to short-term memory recall. Immediately following this testing period, subjects had their electrodes removed and were allowed to leave the laboratory.

One day after initial participation, subjects returned to the laboratory at 2pm, approximately 24 hrs. after initial training, completed the final SSS and were tested on the final 20 pairs. (A_3/V_3). This test measured pre-learning sleep's contribution to long-term memory consolidation. Subjects were then debriefed, credited, and thanked for participation (see **Figure 3.1** for protocol summary).

Results

Performance on the bimodal paired-associates task was assessed as a baseline measure 10 minutes after the learning session (Test 1), after a one-hour retention interval (Test 2), and again after a 24-hour retention interval (Test 3). These measures were taken to first ensure that both groups learned similarly and then to assess the effects of sleeping prior to learning on both short-term retention and long-term consolidation of the learned material, respectively. Subjects were divided into one of two conditions (Wake, n = 12; Nap, n = 13) and either slept or remained awake before being trained on the paired-associates task to a minimum 75% criterion level, to ensure similar encoding between groups. Performance on the bimodal paired-associates task was calculated by dividing the corrected recalled words during testing, which had been paired to the cued sound clips during learning, by the total number of possible correct answers to arrive at a percentage correct score for each test session (see **Table 3.1** for summary). Answers that were incorrect, yet related or similar to the target word (e.g. castle vs. palace, figure vs.

finger), were given half credit.

Sleepiness Measures

The Stanford Sleepiness Scale uses a numerical scale 1-7 (1 being least sleepy, 7 most) to rate levels of alertness/sleepiness. Participants completed three SSS scales, immediately before beginning the learning session at approximately 1:45pm, before the second test session occurring after the one-hour retention interval at approximately 3:45pm, and again one day later before the final test session at approximately 2:00pm. Group means ± SEM for each measure were, respectively: Wake = 2.67 ± .225, 2.92 ± .193, 2.00 ± .213; Nap = 2.46 ± .183, 2.46 ± .215, 1.85 ± .222. There were no group differences on these subjective ratings using 2-tailed independent t-tests at any session (SSS1 $t_{23} = .712$, $p = .483$; SSS2 $t_{23} = 1.56$, $p = .131$; SSS3 $t_{23} = .498$, $p = .623$).

Bimodal Paired-Associates Memory

All subjects were trained to a minimum criterion level of 75% while those who scored higher than 95% during learning were excluded to avoid a ceiling effect. Mean percentage scores during the learning session for each group ± SEM were as follows: Wake = 80.25 ± 2.58, Nap = 79.08 ± 2.46. An independent t-test revealed that the groups encoded the information similarly ($t_{23} = .329$, $p = .745$). The number of trials necessary to reach criterion for each group was calculated and the mean ± SEM for each group was: Wake = 2.92 ± .288, Nap = 2.46 ± .144. An independent t-test revealed that the groups were not significantly different on this as well ($t_{23} = 1.45$, $p = .161$).

Using an independent t-test to analyze performance at Test 1, baseline performance of both groups, which was measured after a brief 10-min retention period after the learning session, was compared. Mean recall percentage ± SEM for each group was: Wake = 82.75 ± 3.57, Nap = 86.54 ± 2.80. No significant difference was found between the groups ($t_{23} = .843$, $p = .408$), indicating that both groups performed similarly at baseline.

In order to measure the effects of having sleep prior to learning new material on short-term retention, change in performance from Test 1 to Test 2 was examined. Again, mean percentage scores ± SEM for Test 1 and Test 2 (Wake = 68.58 ± 2.80, Nap = 79.00 ± 2.95) was the dependent variable. Repeated-measures ANOVA, with Condition (Wake, Nap) as the between-subject factor and Time (Test 1, Test 2) as the within-subject factor, revealed a highly significant main effect of Time ($F_{1,23} = 33.56$, $p < .001$) indicating that, in both groups, paired-associates memory degraded over a waking interval of one hour. A trend towards significance in the interaction between Time and Condition was found ($F_{1,23} = 3.13$, $p = .090$), indicating that the performance of the Wake group degraded to a greater degree than Nap groups' over the short waking retention period, with performance at Test 2 significantly different between groups ($t_{23} = 2.55$, $p = .018$) (**Figure 3.2**).

Change in performance from Test 1 to Test 3 was examined in order to measure the effects of pre-learning sleep on long-term consolidation of the paired-associates. Test 3 performance, mean recall percentage ± SEM, for each group was: Wake = 50.42 ± 3.45, Nap = 71.54 ± 2.74. Using repeated measures ANOVA, with Condition (Wake, Nap) as the between-subject factor and Time (Test 1, Test 3) as the within-subject factor, a highly

significant main effect of Time was revealed ($F_{1,23} = 120.49$, $p < .001$). This finding indicates that both groups' performance deteriorated over the one-day retention period regardless of the subjects having slept or remained awake before learning. Also found was a significant interaction between Condition and Time ($F_{1,23} = 16.16$, $p = .001$), revealing that the Nap group retained the learned material from Test 1 to Test 3 better than the Wake group ($t_{23} = 4.83$, $p < .001$) indicating that having sleep prior to learning new material facilitates long-term consolidation (**Figure 3.3**). Repeated measures ANOVA was used to analyze change in performance across the duration of the experiment, within-subject factor of Time (Test 1, Test 2, Test 3) for both conditions (Wake, Nap). A highly significant main effect of Time was revealed ($F_{2,46} = 92.04$, $p < .001$) indicating the decline in performance over time regardless of condition. A highly significant interaction was also found between Condition and Time ($F_{2,46} = 12.54$, $p < .001$), showing preserved memory in those who napped versus those who remained awake (**Figure 3.4**).

Sleep Data

The PSG recordings were scored for the Nap group according to Rechtschaffen and Kales standard scoring criteria. Total sleep time (TST), sleep latency, stages 2, 3, and 4, slow wave sleep (stages 3+4), and REM sleep were calculated. Mean ± SEM in minutes for each parameter was: TST = 46.27 ± 3.19, latency = 7.31 ± .674, S2 = 21.11 ± 1.75, S3 = 12.88 ± 1.58, S4 = 5.92 ± 1.68, SWS = 18.81 ± 2.25, REM = 2.12 ± .876. Correlation and regression analyses were conducted to determine if any particular stage

of sleep could predict or explain performance in the sleep group. However, no significant statistics were found for any parameter in these analyses.

Discussion

The present study investigated the effects of a pre-learning nap versus remaining awake prior to learning on subjects' encoding efficiency, short-term retention, and long-term consolidation of bimodal paired-associates. Given that previous research, either directly examining pre-learning sleep or including learning before and after sleep as components of the design, has reported conflicting findings, giving sleep either a detrimental or beneficial role, or no role at all, the experimental design here was simplified to address the question. Using a napping protocol to eliminate deprivation effects and implementing a variation on a known hippocampal-dependent declarative memory task, support was found for the hypotheses that pre-learning sleep facilitates subsequent short- and long-term processing of the learned material compared to an equal amount of time spent awake.

While group differences were seen, although only trending toward significance, after an hour retention period, highly significant differences emerged after one day. Still an unanswered question is whether the memory trace in the group who obtained the nap was actively preserved compared to the wake group, or whether not having a nap further degraded the memory compared to those who slept. The former explanation fits best in this experiment since the napping protocol involves no sleep deprivation and the negative consequences of that brain state compared to a normal rested brain. Rather, we are witnessing the positive influences of attaining additional sleep during the day compared

to a normal waking state. It is unlikely that differences in fatigue or attention contributed to the present findings, as no significant differences were found between the groups on any of sleepiness measures. Because there is relatively little literature from which to glean a mechanistic explanation for this type of pre-learning nap benefit, one can only hypothesize about how sleep facilitated consolidation in this scenario.

It is likely that consolidation happens to some degree while awake through time-dependent consolidation, although it is generally accepted that post-learning sleep actively facilitates this process on a faster time scale. The best-fit theory that can explain how sleeping *prior* to learning influences consolidation is Tononi and Cirelli's Synaptic Homeostasis Hypothesis. Using this theory's framework, one would expect that synapses are downscaled during the nap, bringing them closer to baseline levels, lowering the firing threshold and restoring the resources necessary to encode new information and further efficiently process it. The new information subsequently encoded into the "reset" brain exists with less competition from previously encoded information, because this has been either consolidated or discarded during the sleep period. Therefore, it is hypothesized that the new information, the paired-associates memory task in the present study, although encoded equally between the groups, is able to be more efficiently and rapidly consolidated during the subsequent waking period through time-dependent consolidation.

An alternative explanation is that, through standard sleep-dependent consolidation, information learned outside of the experiment was consolidated during the experimental nap, shifting the burden of reactivation from the hippocampus and, again freeing up the hippocampal neurons to take on new information. This particular theory

was adopted by Mander et al. (2011), when justifying their similar results, over the homeostasis theory due to their inclusion of a non-hippocampal dependent procedural task. Over the experimental day they saw no difference between the nap and no-nap groups in how well they were able to learn the procedural task. Because homeostatic synaptic downscaling is thought to occur brain-wide, specifically in brain areas involved in the learning task (Huber et al., 2004), Mander et al. hypothesize that both procedural and declarative memory would benefit from it. However, the present study only investigated declarative learning and, therefore, either theory can account for the findings here.

While this study was able to demonstrate a sleep benefit to subsequent processing of paired-associates, no significant difference was found between the groups in encoding efficiency, with both groups requiring a similar number of trials to learn the material to criterion and both groups performing equally at baseline. A learning criterion was used in our task in order to ensure equal encoding to rule out that group differences over time were simply due to one group learning the material better than the other. However, it was anticipated that a difference in the number of trials needed to reach 75% correct would emerge. That this wasn't found further justifies the conclusion that sleepiness or lack of attention confounded the results, since these factors would also affect how efficiently the material was encoded. It was hypothesized, based on the homeostasis theory, that the group remaining awake would require more trials to encode due to taxed synaptic resources. It could be that the task was not difficult enough to bring forward such differences, but that, once the material was encoded, the differences in the downscaled brain and the taxed brain emerged while consolidating the memory from short- to long-

term stores. However, in Study II, such encoding differences *were* found between groups that had slept or remained awake prior to learning, using the same bimodal paired-associates task. In that study, subjects were trained on the task before a nap/no-nap interval and then trained on a stimulus-related interference task after the retention interval, which required subjects to pair new words with sounds that had previously been paired to the originally learned words. The key difference between the post-sleep encoding scenarios in that study and the present one is that, in this study, subjects were encoding the paired-associates for the first time, forming new synaptic connections representing the memory, while, in Study II, subjects were most likely modifying already potentiated networks representing the pairs, updating them with the new word/old sound information. This modification of already taxed synaptic networks in the waking group potentially explains the significantly greater number of trails necessary to learn the interference task in that study.

Whether through homeostatic downscaling or standard sleep-dependent consolidation, it is clear that pre-learning sleep plays a role in preparing the brain to take on and subsequently process new material. The results of this nap study lends further support to pre-learning deprivation experiments with similar findings, helping to justify the conclusions as related to memory processing and not strictly fatigue effects.

Overall Conclusions

The current series of studies demonstrates the facilitating effect of both pre- and post-learning daytime naps on declarative memory, both recognition and associative memory. Exploration into the temporal relationship between learning and sleep has been largely neglected in the literature, with evident shortcomings in the examination of when the period of sleep needs to occur in relation to learning, how much and of what type of sleep is necessary to facilitate a memory trace, and how pre-learning sleep prepares the brain for new information. Clearly the type of declarative memory task used plays a role in answering these questions. Study I used a recognition task with neutral pictures and did not find the frequently reported facilitation by immediate sleep, whereas Study II, using a more robustly hippocampal dependent paired-associates task, did. This unexpected finding opens the door for future research examining processing the recognition of neutral material during wake or a potential optimal time window for this type of declarative consolidation. It also makes clear the need to delve further into the underlying physiological nature of different types of memories, even within already well-defined declarative episodic and semantic memory.

The results of all three studies conclusively and exclusively lend support to the idea that sleep, with its ideal neurochemical and physiological properties, plays an active role in the strengthening and preservation of declarative memories, rather than a passive or permissive role. Being behavioral studies, one can only hypothesize as to the mechanisms and phenomena involved in such active processing, but it is clear that simply the act of being in a state of sleep, protected from outside interference, is not enough to

explain the current findings. This is an important contribution to a hotly debated topic and could possibly inspire future research along these lines to further support the *active* theory.

While direct correlations between performance and any one sleep stage were not evident within the framework of our findings, slow wave sleep (SWS) emerges as the most influential to the stabilization, protection, and strengthening of the memories in this series of studies based on widely accepted mechanistic explanations. Study I positively correlated the length of the delay before sleep with both performance and the amount of SWS achieved in a nap, indirectly suggesting that perhaps better performance was related to more SWS, however high variability in both measures prevented a direct correlation. Study II found that SWS must be achieved, rather than simply sleep onset and earlier stages 1 and 2, in order to see superior short-term retention, protection from subsequent related interference, and long-term consolidation. No correlation was found, again, between amount of SWS or total sleep time (TST) and performance, suggesting that, perhaps, one only needs to enter deep sleep in order to begin the consolidation process that could carry through after initiation. If it was simply that subjects slept longer in the 60-min nap group compared to a 10-minute nap, then we would expect to see benefits to memory proportional to amount of time slept, and no such correlations were found. Therefore, it can safely be suggested that SWS is key in these findings. The results from Studies I and II inform the findings of Study III, which, standing on its own, can only conclude that sleep prior to learning is better than no sleep. Taking the findings of the first two studies, it is reasonable to assume that SWS also plays a vital role in preparing

the brain to take in and process new declarative information, either through consolidation or homeostatic downscaling during pre-learning sleep.

The series of studies here were able to achieve significant results with the application of 60 to 90 minutes of daytime sleep. This napping protocol was implemented in order to reduce circadian and deprivation effects often seen in overnight sleep protocols. However, it is unknown whether the findings that emerged after napping would be greater with a full night protocol. As it is, the fact that significance was able to be achieved in such an economic fashion is impressive and has great implications to society as a whole. It is becoming ever more acceptable to sacrifice sleep in the name of productivity, unfortunately to the detriment of health and cognitive functions. Studies such as these can inform the community of the need for sleep for optimal performance, both in the workplace and out.

Table 1.1 Summary of sleep parameters across nap groups (mean ± SEM, in minutes)

Condition	TST	S2	SWS	REM	Latency
Immediate	78.11 ± 5.45	43.33 ± 5.64	9.67 ± 3.63	15.22 ± 6.23	9.17 ± 2.42
2-Hour	74.94 ± 8.46	34.75 ± 4.97	18.13 ± 4.06	11.94 ± 3.90	6.12 ± 1.45
4-Hour	76.44 ± 6.90	38.28 ± 3.20	20.89 ± 4.06	10.00 ± 3.56	7.20 ± 2.00

One-way ANOVA	F=.051, p=.950	F=.829, p=.449	F=2.32, p=.121	F=.316, p=.732	F=.566, p=.576

Table 1.2 Percentage of correctly recognized previously viewed pictures at Test and Retest across groups (mean ± SEM)

Condition	Corrected Test Performance	Corrected Retest Performance
Wake	77.56 - 1.29 = 76.27 ± 2.52	61.89 - 2.05 = 59.84 ± 3.68
Immediate	82.22 - 1.93 = 80.29 ± 3.35	63.22 - 1.69 = 61.53 ± 4.84
2-Hour	82.22 - 1.92 = 80.30 ± 3.73	69.89 - 1.02 = 68.87 ± 3.84
4-Hour	85.78 - 3.82 = 81.96 ± 2.22	79.78 - 1.00 = 78.78 ± 3.77

One-way ANOVA	F=.644, p=.593	F=4.51, p=.009
Repeated-Measures ANOVA	F=3.19, p=.037	

* Reported in table as average raw performance percentage minus false alarm percentage

Table 2.1 Percentage of correctly recalled bimodal paired-associates during testing session across groups (mean ± SEM)

Condition	Wake	10-min Nap	60-min Nap
Learning	77.58 ± 1.58	80.75 ± 1.57	81.33 ± 2.29
Trials to Criterion	2.50 ± .261	2.67 ± .225	2.08 ± .193
Test 1 *	75.83 ± 3.58	84.17 ± 2.88	90.00 ± 2.13
Interference Learning	93.33 ± 2.84	95.42 ± 1.68	96.67 ± 1.42
Trials to Int. Criterion **	2.67 ± .188	2.08 ± .083	1.75 ± 179
Test 2 (A_2V_2) ***	57.08 ± 3.51	60.42 ± .234	75.83 ± 2.03
Test 3 ******	37.50 ± 6.85	37.50 ± 3.05	60.00 ± 2.75

* One-way ANOVA $F_{2,33} = 5.93$, $p = .006$
** One-way ANOVA $F_{2,33} = 8.67$, $p = .001$
*** Repeated-measures ANOVA (Test 1 to Test 2) $F_{2,33} = 3.78$, $p = .033$
**** Repeated-measures ANOVA (Test 1 to Test 3) $F_{2,33} = 3.78$, $p = .033$

Table 2.2 Summary of sleep parameters across nap groups (mean ± SEM, in minutes)

Condition	10-min Nap	60-min Nap
TST	10.75 ± .305	52.50 ± 2.66
Latency	6.21 ± .667	7.67 ± 1.33
Stage 2	8.21 ± .570	28.08 ± 2.37
Stage 3	0	10.38 ± 1.70
Stage 4	0	3.25 ± 1.48
SWS	0	13.63 ± 2.47
REM	0	4.00 ± 1.54

Table 3.1 Percentage of correctly recalled bimodal paired-associates during testing session across groups (mean ± SEM)

Condition	Wake	Nap
Learning	80.25 ± 2.58	79.08 ± 2.46
Trials to Criterion	2.92 ± .288	2.46 ± .144
Baseline Test 1	82.75 ± 3.57	86.54 ± 2.80
Test 2 *	68.58 ± 2.80	79.00 ± 2.95
Test 3 **	50.42 ± 3.45	71.54 ± 2.74

* Repeated-measures ANOVA (Test 1 to Test 2) $F_{1,23} = 3.13$, $p = .090$
** Repeated-measures ANOVA (Test 1 to Test 3) $F_{1,23} = 16.16$, $p = .001$

Figure 1.1

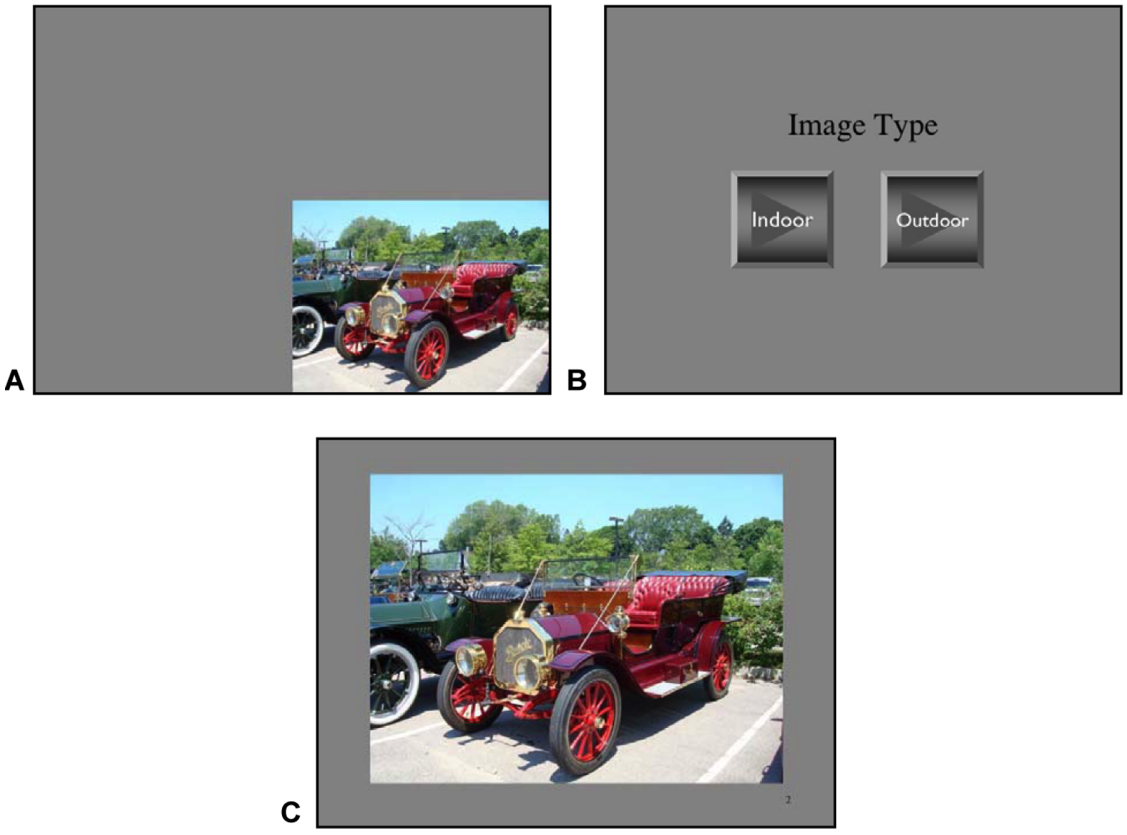

Figure 1.1 Declarative Visual Recognition Task Presentation: Each picture presentation began with a crosshair fixation screen, presented for 1s to direct attention to the center of the screen. A) Individual neutral pictures were presented for 3s in one of four quadrants of the computer screen. B) To ensure attention is being paid, participants were required to indicate whether the picture just viewed was an indoor or outdoor scene by clicking the correct button with the mouse, consequently advancing the slide show. C) During each testing session, pictures were presented mid-screen and participants were required to make "old/new" and spatial location decisions.

Figure 1.2

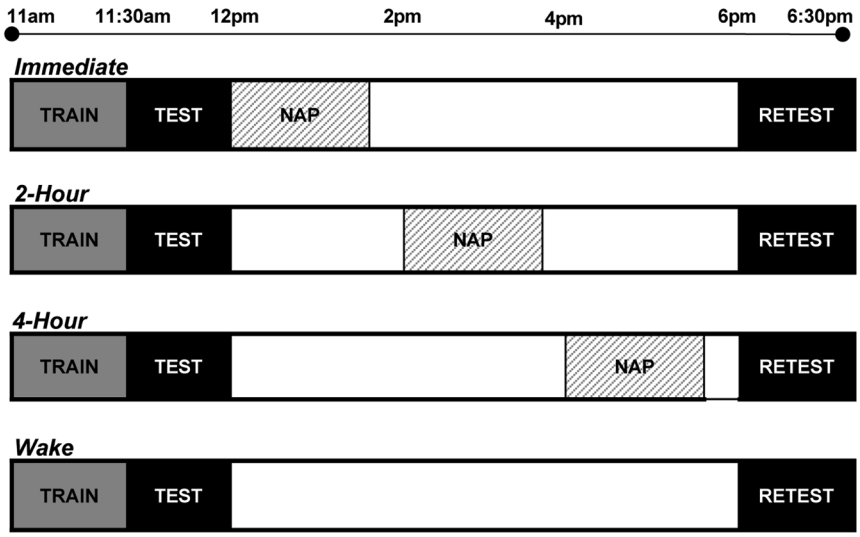

Figure 1.2 Summary of Experimental Protocol: Three experimental groups and the wake control group were all trained at 11am on the declarative memory recognition task, followed immediately by a testing session on a portion of the previously viewed stimuli intermixed with new pictures. After the test phase, sleep groups napped at staggered intervals, either immediately after testing at 12 noon, at 2pm, or at 4pm, while the control group remained awake. All groups were then retested at 6pm on the remaining stimuli, again intermixed with new pictures.

Figure 1.3

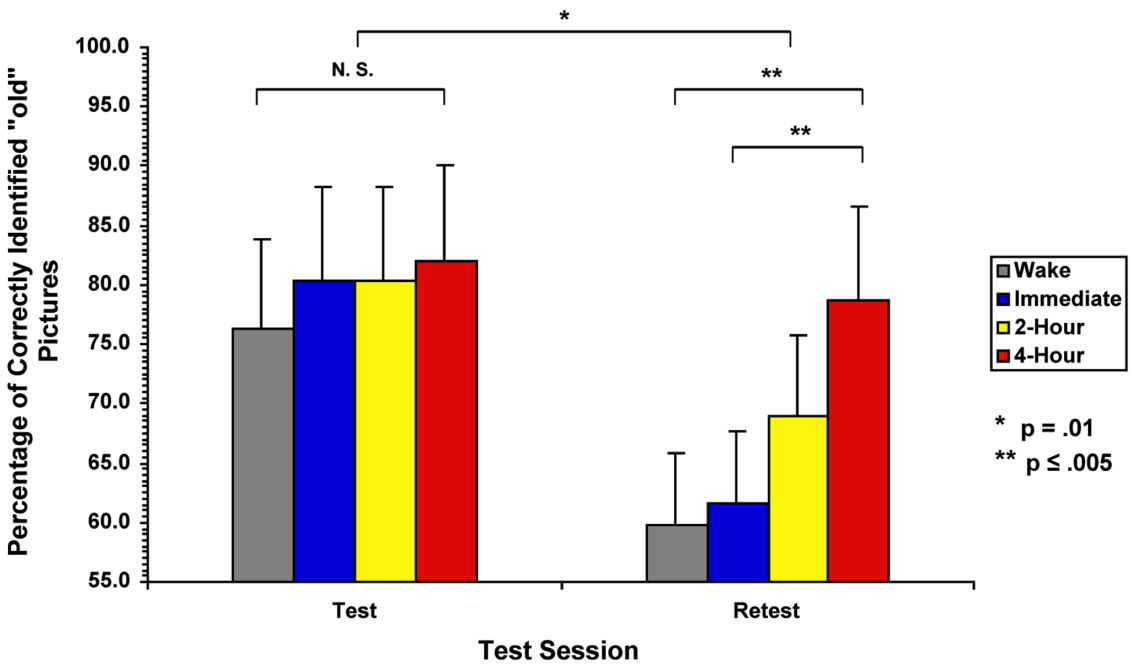

Figure 1.3 Corrected Performance on the Recognition Task at Test and Retest: The y-axis represents the percentage of correctly recognized previously viewed, or "old", pictures, corrected for false alarms. The x-axis represents the scores for the control wake group (Wake) and the three experimental nap groups (Immediate, 2-Hour, and 4-Hour) for the Test and Retest sessions. At Test, all subjects performed similarly, with no significant differences found between the four groups. At Retest, the 4-Hour group performed significantly better than the Wake group (p=.002) and Immediate group (p=.005). Change in performance from test to retest was significant (p=.010), reflecting the interaction between group and test session.

Figure 1.4

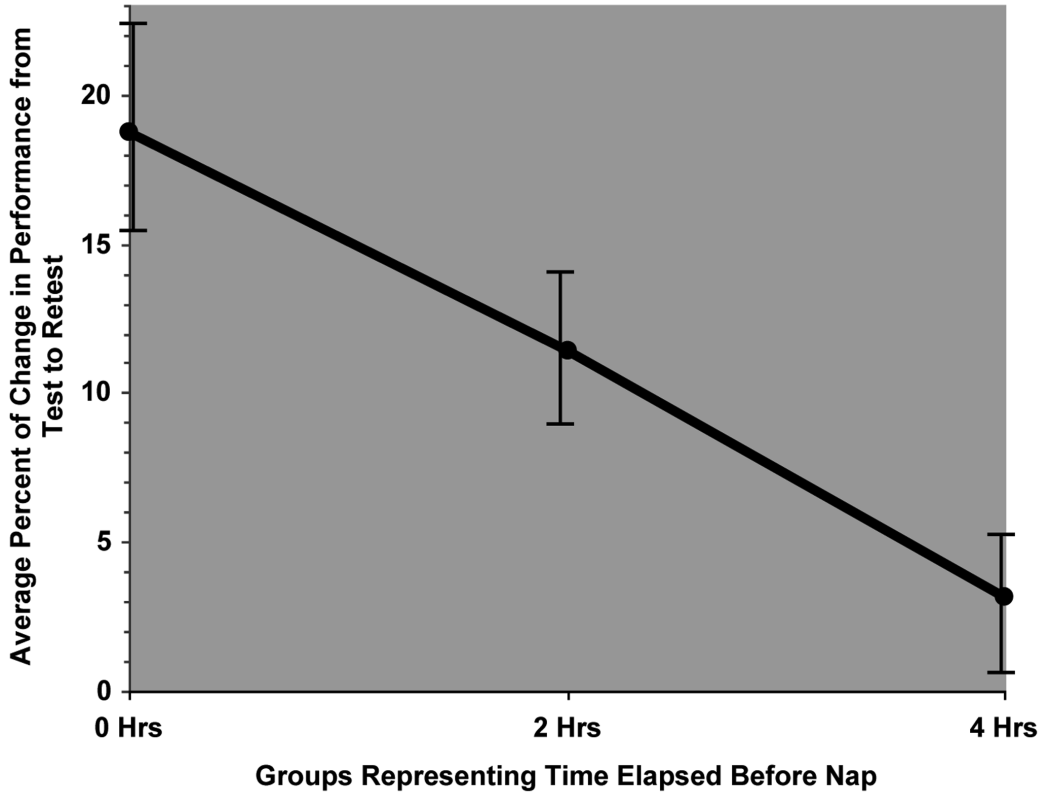

Figure 1.4 Spearman Correlation between Change in Performance from Test to Retest and Groups Representing Time Elapsed before Nap: The y-axis represents the change in percentage of correctly recognized previously viewed, or "old", pictures corrected for false alarms. The x-axis reflects the average amount of time elapsed before napping, at 0 hrs, 2 hrs, and 4 hrs post-learning, representing the Immediate, 2-Hour, and 4-hour groups' performance. Spearman's Rank Correlation revealed a significant negative correlation ($p=.001$), demonstrating that the 2-Hour group follows the trend of better performance with more time elapsed. Correlation was based on individual's change scores. Error bars indicate standard error of the mean.

Figure 2.1

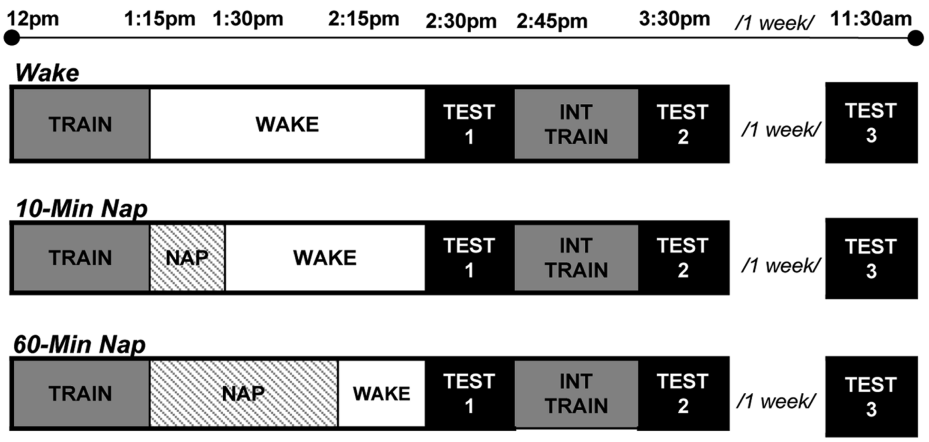

Figure 2.1 Summary of experimental protocol: Two experimental groups and the wake control group were all trained at 12pm on the declarative bimodal paired-associates task to a criterion level of 75%. After the training phase, the nap groups immediately slept, either for a short 10-min nap or a longer 60-min nap, while the control group remained awake. At 2:30, all groups were tested by cued recall on 10 of the learned pairs (A_1V_1) and then immediately trained on the interference task. 10 minutes after training, subjects were tested by cued recall on both original and interfering pairs (A_2V_2, V_{int}). One week after initial learning, subjects were again tested on remaining learned pairs (A_3V_3).

Figure 2.2

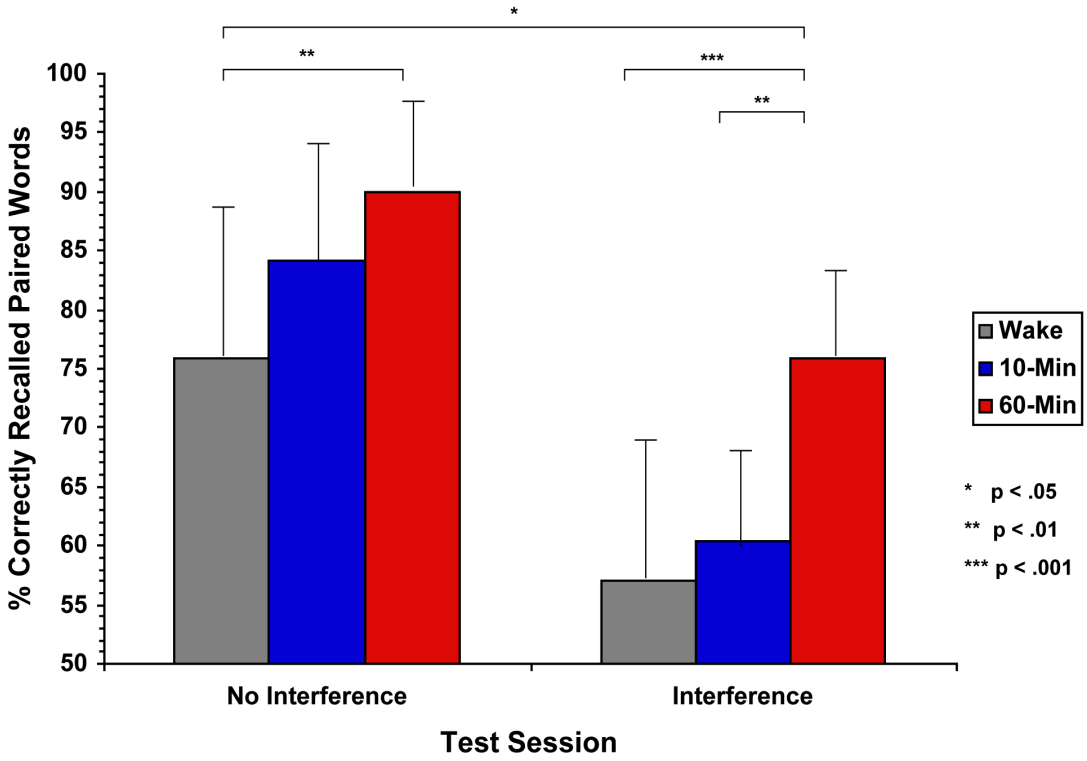

Figure 2.2 Performance on the Bimodal Paired-Associates Task without (Test 1) and with (Test 2) Interference: The y-axis represents the percentage of correctly recalled words paired with cued sounds during testing. The x-axis represents performance for the two nap groups and Wake control groups during Test 1, after the nap/no nap retention interval and before training on the interference task, and Test 2, which measured post-interference recall of originally paired words. Performance at Test 1 was significantly different between groups ($p = .006$), with the Wake group performing significantly worse. There was a significant interaction between Condition and Interference, with change in performance as a result of stimulus related interference significantly different between groups ($p = .033$). Post hoc analysis revealed that the 60-min Nap group performed better than both the 10-min Nap ($p = .006$) and Wake ($p < .001$) groups.

Figure 2.3

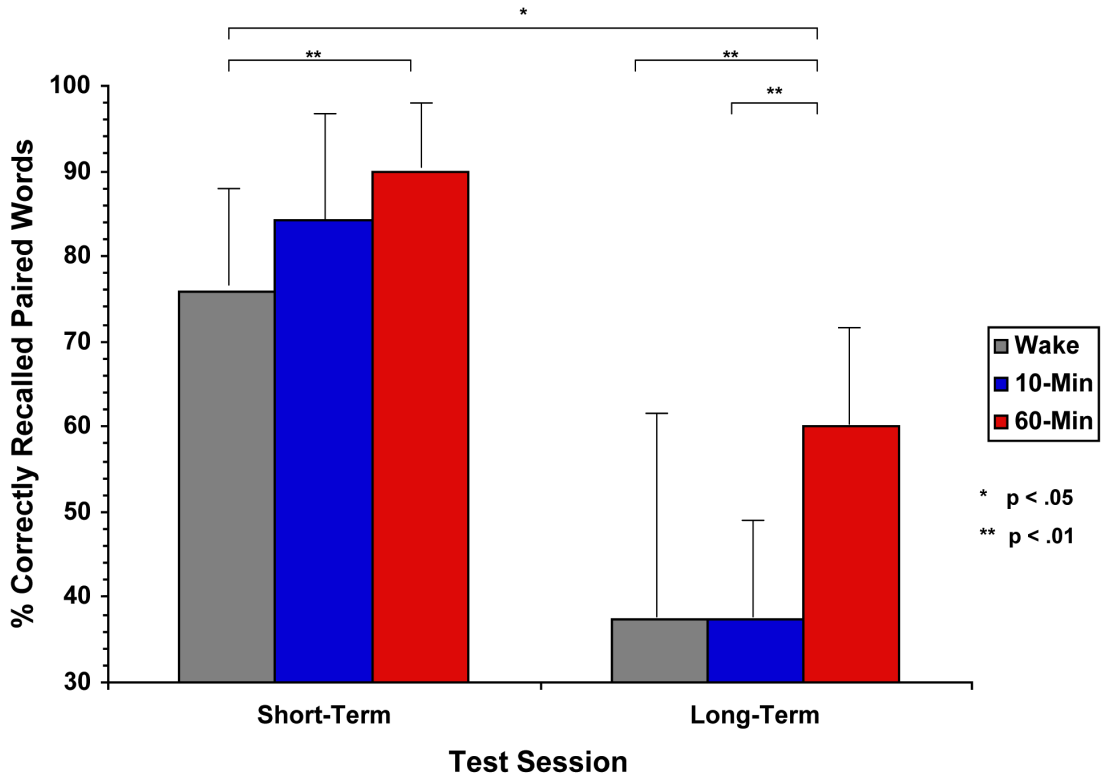

Figure 2.3 Performance on the Bimodal Paired-Associates Task from Test 1 to Test 3 Reflecting Long-Term Consolidation: The y-axis represents the percentage of correctly recalled words paired with cued sounds during testing. The x-axis represents performance for the two nap groups and Wake control groups during Test 1, after the nap/no nap retention interval, and Test 3, one week after initial learning. Performance at Test 1 was significantly different between groups ($p = .006$), with the Wake group performing significantly worse. There was a significant interaction between Condition and Time, with change in performance over a one-week retention interval significantly different between groups ($p = .033$). Performance at Test 3 was significantly different between groups, with the 60-min Nap group performing better than both the 10-min Nap ($p = .002$) and Wake ($p = .002$) groups.

Figure 3.1

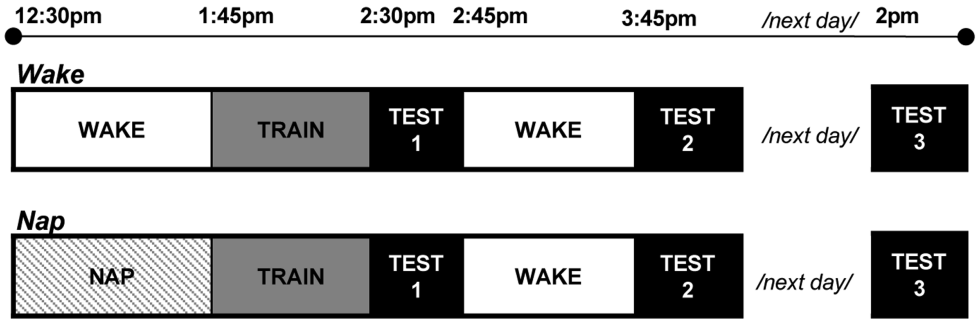

Figure 3.1 Summary of experimental protocol: At 12:30pm, subjects were divided into one if two groups and either napped for 60 minutes or remained awake. At 1:45pm, all subjects were trained on the declarative bimodal paired-associates task to a criterion level of 75%. 10 minutes after reaching criterion, subjects were tested by cued recall on 20 of the learned pairs (A_1V_1) to serve as a baseline measure. All subjects then passively watched nature videos for a 1 hr retention period and were then tested by cued recall on 20 more of the learned pairs (A_2V_2). One day after initial learning, subjects were again tested on remaining learned pairs (A_3V_3).

Figure 3.2

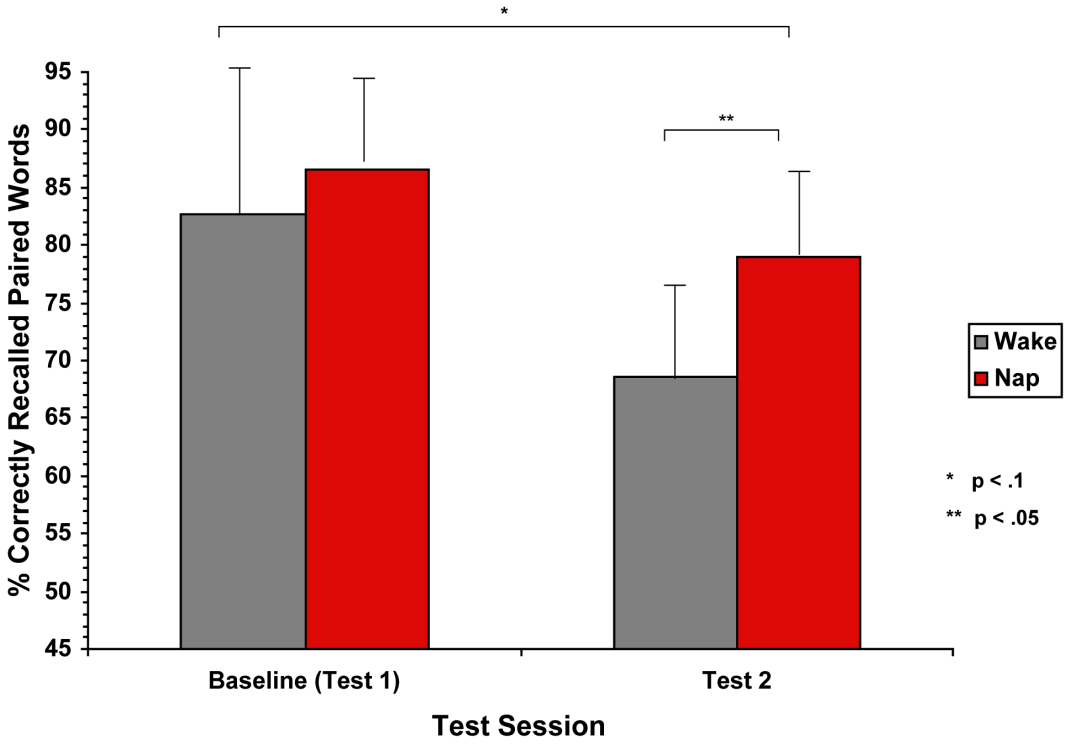

Figure 3.2 Performance on the Bimodal Paired-Associates Task from Test 1 to Test 2 Reflecting Short-term Retention: The y-axis represents the average percentage of correctly recalled words paired with cued sounds during testing. The x-axis represents the performance for Baseline and the Test 2 session after a one-hour waking retention period for both the Wake and Nap groups. There was no significant difference between the groups at baseline, meaning all subjects encoded the material similarly. Change in performance from baseline to Test 2 was trending to significance ($p = .09$), with performance at Test 2 significantly different between the Wake and Nap groups ($p = .018$).

Figure 3.3

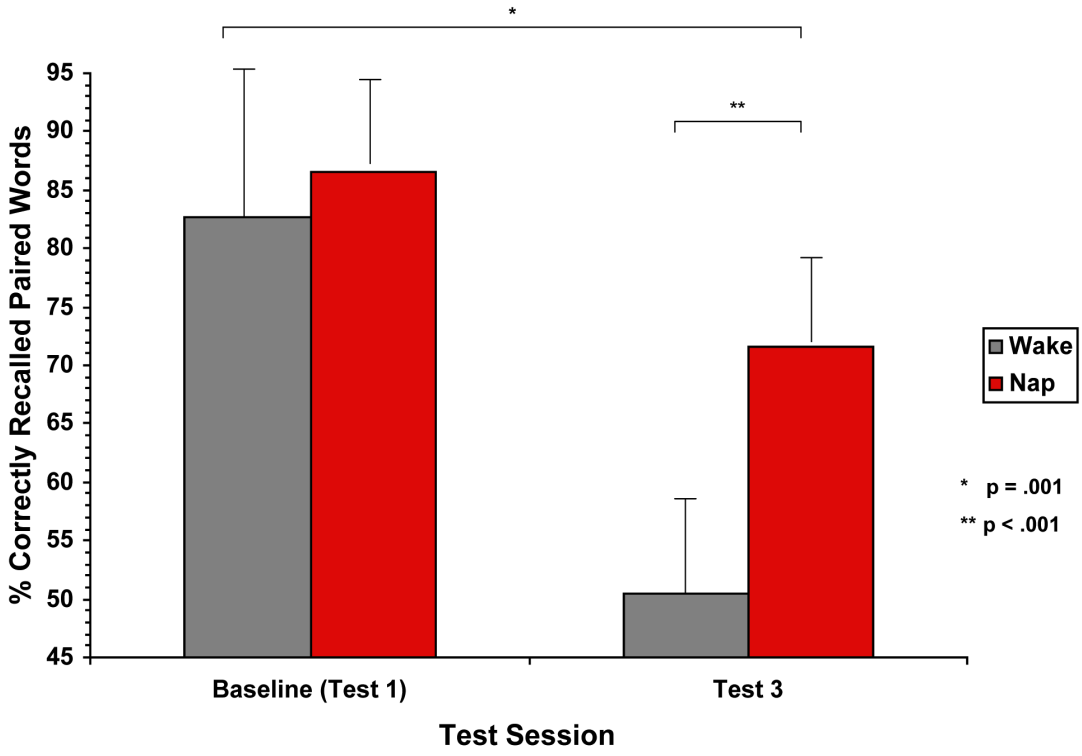

Figure 3.3 Performance on the Bimodal Paired-Associates Task from Test 1 to Test 3 Reflecting Long-Term Consolidation: The y-axis represents the average percentage of correctly recalled words paired with cued sounds during testing. The x-axis represents the performance for Baseline and the Test 3 session after a one-day retention period for both the Wake and Nap groups. There was no significant difference between the groups at baseline, meaning all subjects encoded the material similarly. Change in performance from baseline to Test 3 was significantly different ($p = .001$), with performance at Test 3 significantly different between the Wake and Nap groups ($p < .001$), demonstrating the facilitating effect of pre-learning sleep for long-term memory consolidation.

Figure 3.4

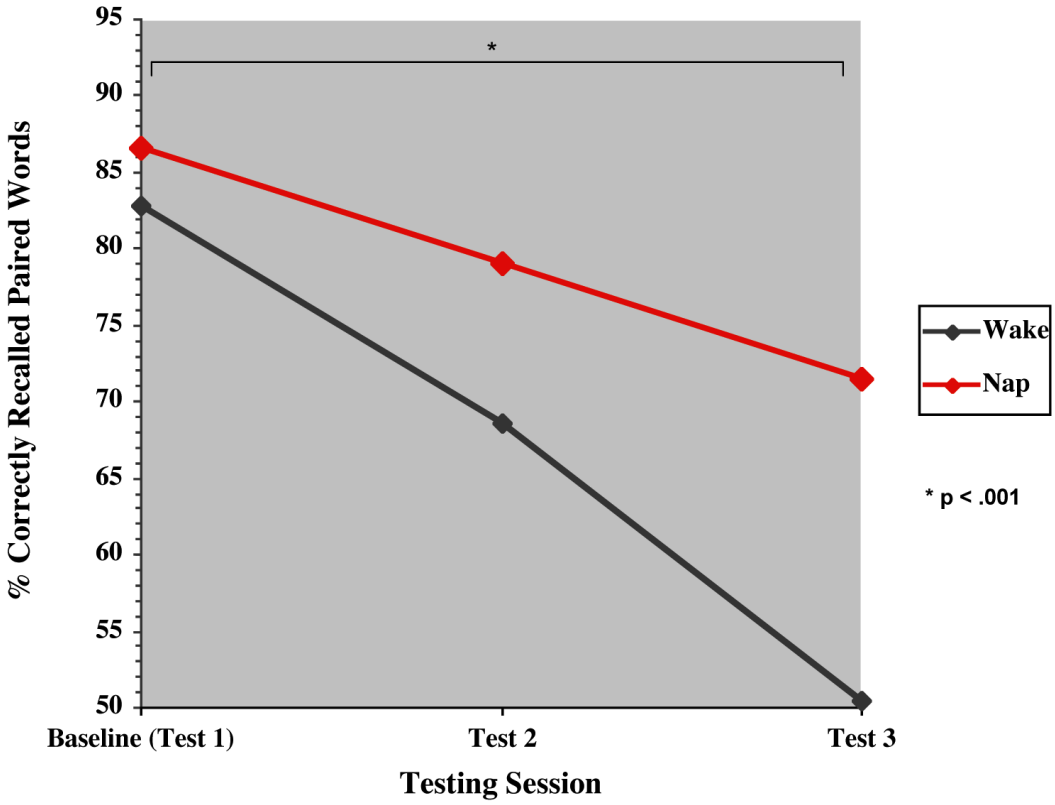

Figure 3.4 Percentage of Correctly Recalled Bimodal Paired-Associates Over Time Across Groups: The y-axis represents the average percentage of correctly recalled words paired with cued sounds during testing. The x-axis represents the performance for all testing sessions over the 1 day experimental protocol for both the Wake and Nap groups. Both groups learned the material similarly, as reflected by the non-significantly different performance at baseline. Performance deteriorated significantly differently between the groups over time ($p < .001$), demonstrating the facilitating effect of pre-learning sleep for long-term memory consolidation.

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