Daytime Napping: Effects on Relational Memory

by

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Abstract

DAYTIME NAPPING: EFFECTS ON RELATIONAL MEMORY

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A plethora of theoretical models and empirical data suggest that sleep strengthens various types of memory. However, the role of sleep in a fundamental feature of memory, relational memory – the flexible representation of items not directly learned prior to sleep – is less clear. At the same time, the effect of daytime naps – relatively brief periods of diurnal sleep – on memory is not well explored. In the present research, a series of three studies were conducted to investigate the effect of daytime napping on three different forms of relational memory: 1) inferential associations of separately learned items, 2) the abstraction of general concepts, and 3) relational memory built on shared contextual elements. Results from all three studies indicate that daytime napping facilitates relational memory. In addition, Study II demonstrates that the effect of daytime napping on relational memory is not dependent on whether the nap immediately follows learning or occurs after a brief (approximately two hours) delay. However, the significant difference in task performance between subjects with and without a nap is not sustained after one week, as shown in Study III. Consistent with the majority of existing literature, slow wave sleep, among all sleep stages, appears to be the strongest contributor to relational memory. Yet it alone cannot fully explain the effect of sleep on relational memory, suggesting that mechanisms independent of sleep stages may be involved. Overall, the results from the present research imply an active role for sleep in multiple memory processes that are not limited to the mere strengthening of memories, but also the binding and reorganizing of separately learned memory traces for flexible use at a later time.

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Overall Introduction

O sleep, O gentle sleep,
Nature's soft nurse, how have I frighted thee,
That thou no more wilt weigh my eyelids down
And steep my sense in forgetfulness?
~William Shakespeare, Henry IV, Part I

I. Sleep facilitates memory

For centuries, sleep has been a subject of rumination for writers, philosophers, and scientists alike. Many, like Shakespeare, posited sleep to either be a state that operates to undo memories formed during wakefulness or a dormant state. However, since the embarkation of systematic investigation on the link between sleep and cognitive functions, scientists showed that sleep participates in many cognitive processes, including one of the most fundamental – memory.

One of the first research studies on sleep and memory (Jenkins & Dallenbach, 1924) reported a facilitating effect of overnight sleep on the recall of nonsense syllables- .audible phonemes without semantic meanings. Other pioneering studies in humans extended the facilitating effect of post-learning sleep to a range of declarative materials, including word lists and paired associates (Yaroush et al., 1971; Fowler et al., 1973). The results were echoed by early animal studies that found similar sleep effects in associative memory tasks (e.g. fear conditioning, passive avoidance) and spatial memory tasks (e.g. maze exploration) (Fishbein, 1970; Fishbein et al., 1971). Reciprocally, some studies showed altered sleep architecture after learning (Fishbein et al., 1974; Smith & Miskiman, 1975). While these early research efforts opened the door to understanding the link between sleep and memory, they had their limitations. The majority limited the investigation on a particular sleep stage - rapid eye movement (REM) sleep and employed selective deprivation methods. Therefore, some studies received criticisms for inability to eliminate confounds such as stress (Albert et al., 1970; Vertes & Siegel, 2005); although most were confound free and provided considerable support for a role of sleep, especially REM sleep, in memory processes._

In recent years, with a growing body of evidence from behavioral and neurobiological studies in both humans (For example: Plihal & Born, 1997; Maquet, 2001; Mednick et al., 2003; Walker et al., 2003; Gais & Born, 2004; Tucker et al., 2006; Gais et al., 2008) and animals (For example: Wilson & McNaughton, 1994; Louie & Wilson, 2001; Lee & Wilson, 2002; Sirota et al., 2003; Fu et al., 2007; Ji & Wilson, 2007), the participation of sleep in memory processes is beyond dispute. In addition, sleep has been linked to gene transcription (e. g. expression of zif-268) important to experience-dependent neural plasticity (Ribeiro et al., 1999), which is widely considered a neural mechanism for formation of long-term memory. Impressively, blockage of cortical activity during sleep prevented cortical synaptic plasticity (Jha et al., 2005), implying an active role of sleep in memory processes.

Thus far, sleep has been linked to many forms of memory, including both implicit types (e.g. perceptual learning, sequence finger tapping, mirror tracing, etc.) and explicit types (e.g. recall of nonsense syllables, pair associates of varying modalities, maze navigation, etc.). However, with the majority of current research centering on how sleep strengthens (Plihal & Born, 1997; Gais & Born, 2004; Tucker et al., 2006) or enhances memory (Mednick et al., 2003; Walker et al., 2003; Gais et al., 2008) of directly learned materials, there has been little attention paid to the relational organization of memory (Eichenbaum, 2004). Ironically, this feature, aptly termed relational memory, may be fundamental to all learning experiences. The present research centers on relational memory arising from direct associative memory of the declarative type. It is important to note that relational memory is different from the conventional definition of associative memory (termed direct associative memory here to make clear the distinction). While the tasks employed in the present research are adaptations of direct associative memory tasks, they probed memory expressions of meta-relationships existing beyond directly learned associations.

II. Relational memory

In real life or in research, manifestation of latent connections beyond directly learned materials is often observed, suggesting deeper processing of the learned materials. While the mechanisms involved are unclear, it has been posited that relational memory is the product of deep processing of direct associative memory (Eichenbaum, 2004). As proposed in several memory models (McClelland, McNaughton & O'Reilly, 1995; O'Reilly & Rudy, 2001; Eichenbaum, 2004) episodic experiences do not exist in isolation, but rather they are integrated into an extended network linked by features common across disparate episodes. The functional consequence of such organization is interconnected memory representations that allow flexible expression when one faces a novel but relevant situation. For example, imagine that a first-time visitor to New York took the D train to go from Brooklyn to Manhattan. Then on a separate occasion, she took the D train again, but this time from Bronx to Manhattan. When she, on her third visit to New York, needed to go from Brooklyn to Bronx, she would probably know to use the D train even without consulting with the subway map. Relational memory does not only enable associations between items learned separately; it may also underlie extraction of rules, generalization, as well as creative application of knowledge – all of which are crucial adaptive behaviors. Although it is likely that differences exist between the initial establishment of relational links and the retention of these links, the existing literature has not distinguished them (Eichenbaum, 2004; Wagner et al., 2004; Ellenbogen et al., 2007; Payne et al., 2009). Similarly, the present work does not attempt to distinguish the two but notes that it is an important question to be addressed in future research.

Relational memory appears to rely heavily on the hippocampus, a brain area important to memory consolidation (Bunsey & Eichenbaum, 1996; Dusek & Eichenbaum, 1997; Davachi & Wagner, 2002; Preston et al., 2004; Dupret et al., 2008; Konkel et al., 2008). Considerable evidence came from studies on rats sustaining selective hippocampal lesion. These studies also provided innovative paradigms to operationalize relational memory. In one particular study, rats learned multiple overlapping odor paired associates (e.g. A goes with B, B goes with C). Normal rats demonstrated two forms of flexible memory expression, symmetry – the ability to associate paired elements presented in the reverse of training order (e.g. B goes with A), and transitivity – the ability to make inferential judgment across stimulus pairs sharing a common element (e.g. A goes with C). Rats with selective hippocampal lesion demonstrated neither form of flexible expression (Bunsey & Eichenbaum, 1996). In another study, rats learned a set of four odor discrimination problems separately (e.g. A > B, B > C, C > D, D > E) with a hierarchy hidden among the overlapping odor stimuli. Animals with the hippocampus disconnected from either its cortical or subcortical pathways succeeded in acquiring the separate discrimination problems but did not demonstrate transitive inference (e.g. B > D) (Dusek & Eichenbaum, 1997).

Likewise, research in humans (Preston et al., 2004; Heckers et al., 2004) and in primates (Lavenex et al., 2007) showed the involvement of the hippocampus in relational organization of memory. Specifically, the hippocampus showed selective activation when subjects had to judge inferentially on indirectly related stimuli compared to directly related stimuli (Preston et al., 2004; Heckers et al., 2004).

Neurophysiological studies also offered complementary evidence. Single unit recording revealed some hippocampal neurons encoding features common to different experiences (Moita et al., 2003; Rivard et al., 2004). This firing pattern is distinct from that corresponding to direct associative memory. For example, in rats, some cells fire only when a stimulus appears in a particular location, while others fire when the stimulus is presented across different trials (Rivard et al., 2004). Hippocampal neurons specifically responsive to meaningful categories of stimuli features similarly exist in monkeys (Hampson et al., 2004) and in humans (Kreiman et al., 2000).

Together, the different lines of research converge on the essential contribution of the hippocampus to the integration of direct associative memory into a relational memory network. It is worth noting that the hippocampus is considered crucial to the processing of declarative memory (i.e. memory one is explicitly aware of) in general, which umbrellas direct associative memory (For a review, see Squire & Zola-Morgan, 1988; Eichenbaum, 2004). This suggests that the difference between direct associative memory and relational memory processes is not the site of processing but rather the level of processing and/or the mechanisms underlying the processes.

III. Sleep and relational memory

Significantly, the hippocampus is active during sleep (Buszaki, 1998; Peigneux et al., 2004; Dang-Vu et al., 2005) and may engage in memory processing in this state (Buszaki, 1998).

For example, the hippocampal place cells that fired in a highly correlated pattern during a food-seeking spatial task exhibited highly correlated firing again during subsequent sleep (Louie & Wilson, 2001; Lee & Wilson, 2002; Ji & Wilson, 2007), suggesting a “reactivation” of memory in sleep. In addition, PET imaging in humans showed increased regional blood flow in hippocampal structures during sleep after being trained in a virtual navigation task compared to untrained subjects (Peigneux et al., 2004). More likely than not, hippocampal activities during sleep reflect ongoing memory processes. An important question would be whether these memory processes include the establishment of a relational memory network. The present research addresses this question with a series of three studies with the task in each study focused on a different form of relational memory.

A handful of sleep studies have used tasks with the characteristics of relational memory, although they used different terminology. One study demonstrated that subjects were more likely to gain “insight” – to learn a hidden shortcut common across trials to solve a number reduction task – after a night of sleep (Wagner et al., 2004). Another study showed sleep promoting false recall of unstudied “critical theme words” semantically related to studied word lists (Payne et al., 2009). A third study, using similar terms as the present research, found “relational memory” – measured by performance on a transitive inference task – to benefit from nocturnal sleep (Ellenbogen et al., 2007). The present research expanded on the limited amount of data on the involvement of sleep in relational memory. It aimed to establish the necessary generalization of the sleep effect to different forms of relational memory. Based on the previous studies, it had adopted the *a priori* hypothesis that sleep would facilitate various forms of relational memory. Furthermore, the present research examined three important questions unanswered by previous research: 1) Does a brief period of sleep, such as a daytime nap, facilitate relational memory? 2)

Does sleep need to immediately follow learning in order to facilitate relational memory? 3) Does post-learning sleep have a long-lasting effect on relational memory?

IV. The daytime napping paradigm

The present research assessed the effect of sleep on the relational memory by using a daytime napping paradigm. This paradigm had only been used in a few published studies (Mednick et al., 2003; Tucker et al., 2006, 2008), all of which found a beneficial nap effect on memory, similar to overnight sleep studies. Moreover, the daytime napping paradigm has several advantages. First, it is devoid of confounds, such as stress and fatigue, commonly associated with sleep deprivation paradigms. Second, it can circumvent circadian factors intertwined with many non-deprivation sleep studies. Typically in these studies, a sleep group that learns at night and is tested in the morning is compared to a wake group that learns in the morning and is tested at night. Lastly, this paradigm has a great deal of practical value. Given its common occurrence and brevity, daytime napping can serve as an effective, easily-implemented facilitator of memory.

In each of the three present studies, nap subjects had an opportunity of about 90 minutes in the afternoon to sleep. This time window allowed collection of an adequate amount of sleep data for analysis as well as allowed subjects an opportunity to cycle through all the sleep stages.

V. Sleep stages and memory processes

Another important aspect of the present research is the question of whether individual sleep stages, characterized by dramatically different physiological features, contribute differentially to memory processes. There are five cyclically progressing sleep stages: stage one sleep (S1) is a transient, light sleep state; stage two sleep (S2) is marked by slower brain activities with occasional burst of brain waves called spindles and K-complexes; stage three (S3) and stage four (S4) sleep are collectively called slow-wave sleep (SWS), featuring namesake slow oscillating brain activities. Lastly, rapid eye movement (REM) sleep is characterized by rapid, low-voltage brain activities similar to those in wakefulness. The majority of existing literature, on both the theoretical (Buszaki, 1998; Hasselmo, 1999; Sejnowski & Destexhe, 2000; Tononi & Cirelli, 2002, 2006) and empirical level (Yaroush et al., 1971; Plihal & Born, 1997, 1999; Walker et al., 2002, 2003; Gais & Born, 2004; Tucker et al., 2006) suggests that the contribution of each sleep stage to memory processes may not be equal and may depend on the type of memory.

Among all of the sleep stages, SWS has been proposed to be particularly important for declarative memory. This proposal gained momentum as the neurophysiology and neurochemistry observed in SWS appear to provide viable mechanisms for the processing of declarative memory. The processing, especially the consolidation, of declarative memory is thought to depend on interactions between the hippocampus and the neocortex (McClelland, McNaughton & O'Reilly, 1995; O'Reilly & Rudy, 2001). In a widely-accepted model of sleep and memory (Buszaki, 1998), the hippocampal-neocortical dynamics are associated with the wake-sleep cycle. Declarative information acquired during wakefulness is temporarily stored in the hippocampus. The information is later "transferred" to the neocortex through hippocampal physiological events, called sharp wave-ripple complexes, during SWS.

A complementary model emphasizes the mnemonic value of fluctuating levels of a neurotransmitter, acetylcholine (Ach), across wake-sleep states (Hasselmo, 1999). Accordingly, the flow of information from the neocortex to the hippocampus is optimized during wakefulness when Ach levels are high, while the reverse is optimized during SWS when Ach levels are at their lowest levels.

The hypothesized role of SWS in declarative memory is supported by empirical data. Behavioral studies reported that declarative memory, specifically direct associative memory, benefited from the early half of nocturnal sleep, rich in SWS (Yaroush et al., 1971; Plihal & Born, 1997), and from a nap containing only non-REM (NREM: S1, S2, and SWS) sleep (Tucker et al., 2006). Notably, these studies suggested a double dissociation of sleep stages with REM sleep selectively facilitating procedural memory (i.e. implicit memory of how to perform motor procedures) but not declarative memory while the opposite is true for NREM sleep. Lending further support to the hypothesis, during SWS, the hippocampus showed neuronal activities reminiscent of that during wakefulness (Wilson & McNaughton, 1994; Lee & Wilson, 2002; Sirota et al., 2003), as well as increased regional blood flow related to learning (Peigneux et al., 2004).

The present research examined relational memory built on direct associative memory. In order to delineate the role of individual sleep stages in memory processes, the present studies used the duration of sleep stages to predict memory performance in regression analyses. In accordance to the majority of existing literature, SWS was expected to be the main contributor to the different forms of relational memory probed here. In fact, Study I experimentally excluded REM sleep in order to better establish a causal link between NREM sleep, specifically SWS, and relational memory. While a significant effect was detected, as expected, data from the excluded subjects (data not reported here), who obtained REM sleep in their nap, indicated a trend of correlation between REM sleep and relational memory.

Careful examination of existing literature suggests this correlation might not be a fluke for several reasons. First, as discussed before the selective facilitation on declarative memory by the early half of nocturnal sleep has been contributed to SWS (Yaroush et al., 1971; Plihal & Born, 1997, 1999). However, while it is SWS-dominated, early nocturnal sleep still contains epochs of REM sleep (not to mention a large amount of S2). Therefore, it would be premature to conclude SWS to be the sole sleep stage involved in the processing of declarative materials. Second, early animal studies consistently linked REM sleep to memory (Fishbein & Gutwein, 1977; Smith & Butler, 1982), even when employing complex associative tasks (Scrima, 1982). Third, imaging studies have also found neuronal reactivation related to episodic experience (one type of declarative memory) in REM sleep (Louie & Wilson, 2001; Peignaux et al., 2003 Maquet, 2004; Hasselmo, 2008). Fourth, and of particular importance, it is still unclear whether the processing of relational memory is exclusive to the declarative or non-declarative memory system, or if it is, perhaps, fundamental to both systems. Two out of the only three studies on sleep and relational memory used tasks with non-declarative elements (Wagner et al., 2004; Ellenbogen et al., 2007). Limiting the analyses to SWS, which may be partial to declarative memory processes, could in fact limit the understanding of relational memory. Therefore, based on these reasons and the preliminary finding from Study I, subjects were subsequently allowed to obtain REM sleep in Studies II and III. Memory performance was regressed on duration of individual sleep stages. Of interest, besides SWS and REM, S2 – characterized by spindle activity – has also been linked to learning and retrieval of memory (Gais et al., 2002; Fogel & Smith, 2006; Eschenko et al., 2006). However, considering the declarative elements in the current tasks and that there is more evidence for the involvement of SWS in declarative memory, the present research operated with the *a priori* hypothesis that SWS would the primary contributor to the different forms of relational memory probed here.

VI. Brief summary of the present studies

The present research consists of a series of three studies, each focusing on a different form of relational memory. Study I, using a visual version of the paired associate task, investigated whether a brief daytime nap would facilitate the indirect relational association of faces commonly linked to the same object. Study II employed a Chinese character learning task to examine the extraction of a general rule. In addition, it addressed the quesion of whether napping needed to immediately follow learning in order to facilitate relational memory. Study III examined the relational link between items sharing common temporo-spatial contextual cues (i.e. items sharing the same presentation order in separate sequences and items sharing the same presentaion location on a screen). At the same time, it investigated whether a brief daytime nap would have a long-lasting effect on this particular form of relational memory.

Study I – Daytime napping: effects on indirect relational associations

Introduction

Ample behavioral and neurobiological evidence indicates that sleep facilitates the processing of declarative memories. However, most research thus far has focused on how sleep strengthens direct associative relationships, as in between two words in a word pair that are directly learned (Plihal & Born, 1997; Gais & Born, 2004; Tucker et al., 2006). Of equal importance, however, is the question of whether sleep can facilitate the formation of indirect associations across events which were not directly learned, but share common features – a form of memory known as *relational* memory.

Relational memory, as a construct, has emerged from several models of declarative memory (McClelland, McNaughton & O'Reilly, 1995; O'Reilly & Rudy, 2001; Eichenbaum, 2004) suggesting that individual episodic experiences do not exist in isolation, but rather in extended networks linked by common features between related memories. These elements are often "arbitrary or accidentally occurring relations among the constituent elements of experience" (Konkel & Cohen, 2009). A functional consequence of such organization is "its support of flexible expression of memory, a quality that permits inferential use of memories in novel situations" (Eichenbaum & Cohen, 2001). In this context, relational memory, distinguishable from direct associative memory, is the ability to make associations between items learned separately. The present study aimed to assess the effect of daytime naps on the processing of this aspect of declarative memory.

In recent years, a case has been built for non-rapid eye movement sleep (NREM sleep, Stages 2, 3, & 4) to be a particularly optimal brain state for declarative memory processing, with slow wave sleep (SWS, Stage 3 & 4) being the key contributor. Slow wave sleep is characterized by physiological and neurochemical properties hypothesized to optimize declarative memory processing during this sleep stage (Buszaki, 1998; Hasselmo, 1999). It is quite possible that these same mechanisms may also be crucial to relational memory, which arises from a declarative memory network. In the present study we investigated whether sleep contributes to relational memory processing and, more specifically, whether individual sleep stages are important for this processing.

Behavioral studies seeking to delineate the unique effects of NREM sleep on memory have adopted two experimental approaches. Some research has taken advantage of the natural progression of nocturnal sleep, in which a preponderance of SWS is observed during the first half of a normal night of sleep, while REM sleep dominates the second half. Declarative memory recall benefits more from early-night SWS-rich sleep, than from late-night REM-rich sleep, or wakefulness (Yaroush, et al., 1971; Plihal & Born, 1997). However, this approach cannot completely separate the influence of NREM from REM sleep as brief periods of REM still occur in early-night sleep. Studying naps, on the other hand, provides a unique opportunity for isolating NREM sleep (i.e., participants are awakened prior to entering REM sleep) (Tucker et al., 2006; Tucker et al., 2008). The nap design also circumvents deprivation-induced stress and circadian factors because participants are not sleep deprived during the day, and they learn and are tested at the same time of the day. Furthermore, it is also of significant practical value to show that a relatively brief period of daytime sleep has the capacity to facilitate memory performance.

The present study examined the effect of a daytime nap on direct associative memory as well as relational memory. These forms of declarative memory were probed through using an adaptation of an associative inference task (Bunsey & Eichenbaum, 1996; Preston et al., 2004), whereby participants learned two separate groups of photographs comprised of face-object pairs in which the objects were common to both lists. At retest, four hours later, relational memory was assessed with a surprise face-face recognition task, in which participants had to associate the two faces that were previously paired with the same object. This was followed by a test of the face-object pairs. It was hypothesized that a daytime nap composed only of NREM sleep, as opposed to wakefulness, would strengthen the retention of direct associative memories (i.e. face-object pairs). More importantly, it was also hypothesized that relational links not directly associated during learning (i.e., face-face memories) would be established during a nap. Because SWS has been implicated in the processing of different types of declarative memory, NREM sleep overall, and SWS in particular, was expected to be correlated with direct associative and relational memory retention.

Methods

Subjects

Thirty-seven subjects (19 in the nap group and 18 in the no-nap group) were originally recruited from the undergraduate population of the City College of New York. Each subject kept a sleep log for a week prior to the study. Any subject having an irregular sleep schedule was excluded from the study. Four subjects were excluded from the data analysis because they entered REM sleep during the nap. Additionally, two subjects, one from each group, were excluded from the analyses because they scored below chance on the delayed test of the face-object associations. One subject's sleep data was missing due to a computer error. This subject was excluded from the sleep data analyses but not from the behavioral analyses. The remaining thirty-one subjects, with an average age of 21.37 ± 5.86 (mean ± SD), were in good health, free of all medications that might either impair or facilitate sleep. There were 7 females and 7 males in the nap group (n=14, mean age = 21.21 ± 1.10), 10 females and 7 males in the no-nap group (n=17, mean age = 22.12 ± 1.83). Subjects were asked to refrain from drinking coffee or alcohol during the 24 hours preceding participation. All subjects had normal or corrected to normal vision. All subjects signed an informed consent and the study was approved by the City College of New York Institutional Review Board.

Tasks

The task was an adapted version of an associative inference task (Bunsey & Eichenbaum, 1996, Preston et al., 2004). Subjects learned two separate sets of black and white photograph pairs, each of a face and a common household object (AB and BC tasks). The objects (B) were common to both lists but paired with two different faces. After either a daytime nap or a period of wakefulness, subjects were tested on a force-choice task (AC task), in which they had to associate faces previously paired with the same object, followed by retests of AB and BC tasks. Photographs of the faces were adopted from Preston et al. (2004). There were 30 photograph pairs in each set. The stimuli included a total of 60 faces and 30 common objects. The sets contained an equal number of male and female face pictures, and all photographs were adjusted to have comparable brightness and contrast. The size of each photograph was 7.62 cm by 7.62 cm. All stimuli and tasks were presented on a 50-cm flat-panel screen using Microsoft PowerPoint.

Procedures

The subjects arrived at 10:30am at the Laboratory of Cognitive Neuroscience and Sleep at The City College of New York. Upon arrival, subjects were asked to sign a consent form explaining the nature of the research, complete a demographics questionnaire, and complete the Epworth Sleepiness Scale (Johns, 1991). The Epworth Sleepiness Scale is a measure of general daytime sleepiness by asking subjects' tendency to fall asleep in various situations. Subjects were then shown around the laboratory to familiarize them with the laboratory environment and the sound-attenuated bedrooms. The subjects then completed three subtests of the Multidimensional Aptitude Battery-II (MAB-II; Jackson, 1998). The MAB-II is a standardized paper-and-pencil test commonly used to assess intelligence. The three selected subtests were arithmetic, picture arrangement, and picture completion, which have the highest correlations with intelligence measured by the Wechsler Adult Intelligence Scale**.** Each subtest took 7 minutes to complete.

At 11:30am, electrodes were applied to all subjects to ensure a similar experience for them. At approximately 12:00pm, subjects learned the face-object pairs, presented on a 50-cm flat-panel screen (approximately 0.6 m from the subjects) using Microsoft PowerPoint.

Subjects learned the two sets of picture pairs (AB and BC pairs). The objects (B) were the common elements shared by the two sets of photograph pairs. All objects were associated with two faces. The sex of the two faces associated to the same object was random. The positions (left or right) of the pictures (face or object) were randomized. The order of presentation was counterbalanced across subjects such that the AB set of face-object pairs was presented first to half of the subjects, whereas the other half learned the BC set first. Each pair was displayed for 2 seconds with a one-second inter-stimulus interval. Subjects were instructed to pay close attention to each picture pair and were told they would be subsequently tested for retention of the face-object pairs. Subjects had two exposures to each of the AB and BC picture sets. This protocol was found to be optimal based on pilot data that yielded a level of retention of the face-object pairs, which was high enough to ensure adequate retention of direct associative memories and the formation of relational memories. Immediately following two exposures to the first set of picture pairs, subjects completed a forced-choice recognition task – the faces were displayed one at a time along with four photographs of objects, one previously paired with the face, the other three serving as distracters (previously paired with other faces, see *figure 1.1*). Subjects selected the object they thought was paired with each face during learning. Subjects were allowed as much time as needed to complete the forced-choice task. Response time was recorded. The same procedure was used for the second set of picture pairs. The entire learning session and the following forced-choice recognition task took 40 to 45 minutes to complete.

Afterward, subjects were randomly assigned to either the nap group or the no-nap group. At 1:00pm, nap subjects were taken to a sound attenuated bedroom for the nap. No-nap subjects were taken to a second bedroom, where they sat in a semi-recumbent position and watched a non-arousing video about marine life while the nap group slept. The video was chosen to minimize cognitive activity and interference with the task stimuli. Nap subjects were monitored online by digital EEG acquisition software (Gamma System-Grass/Telefactor^{tm}) using a five-channel polysomnographic montage, which included electroencephalography (EEG recorded between electrode pairs C3-A2 and C4-A1), electro-oculography (EOG), and chin-electromyography (EMG) channels. Nap subjects were permitted to sleep for up to approximately 90 minutes. If subjects obtained SWS during this time they were allowed to sleep until it appeared that the SWS period was coming to an end. Subjects were awakened only from sleep stages 1 or 2. Therefore the 90-minute window to nap was slightly expanded for some subjects to accommodate the procedure of waking up subjects. Subjects that entered REM were excluded from data analysis. The sleep recordings were scored using the international criteria of Rechtschaffen and Kales (1968).

At the end of the nap period, the nap and no-nap subjects exited their respective bedrooms and electrodes were removed. Subjects then watched videos until the testing session.

At 4:30pm all subjects were tested, using the forced-choice test paradigm described earlier. During this testing session subjects were first given an unexpected forced-choice retention task on the relational face-face (AC) pairs. For this task, a cue face (A) was shown along with four face choices, one correct match (C, the face previously associated with the same object B) and three distracters (faces learned but paired with different objects, see *figure 1.1*). The position of the correct face in the display of four faces was random. The AC pairs were always tested first, followed by forced-choice retention on the AB and BC pairs.

Results

Assessment of AB and BC face-object retention immediately after learning (number of correct responses) were labeled as AB1 and BC1 scores. Assessment of AB and BC face-object retention at testing were labeled as AB2 and BC2 scores. Relational memory was measured by number of correctly matched face-face pairs – AC scores.

All analyses assumed two-tailed alpha level of 0.05.

Sleepiness and Intelligence Measures. There was no group difference in the Epworth Sleepiness Scale scores (Independent samples t-test, $p = 0.33$).

Intelligence measured by the three MAB-II subtests – arithmetic, picture arrangement and picture completion – were standardized. Z-scores were calculated and used in the following analyses. The two groups demonstrated similar performance on all three measures of intelligence (Independent samples t-tests, all p values > 0.50).

For a summary of group means and SEMs of these measures, see *table 1.1*.

Response time. Subjects' average response time was 5:55 ± 1:19 (min:sec, mean ± SD) for the AB1 task, 6:03 ± 1:41 for the BC1 task, 5:27 ± 1:14 for the AB2 task, 5:22 ± 1:30 for the BC2 task, 9:52 ± 2:43 for the AC task, respectively. Although subjects were allowed as much time as needed, no subject used more than 16 minutes. Maximum response time was 15 minutes and 45 seconds. Subjects took considerably more time to complete the face-face task than any of the face-object tasks (Paired t test, all p values < 0.05). No significant difference in response time was found between the groups on any of the tasks (Independent samples t-tests, all p values > 0.10, see *table 1.2* for means and SEMs).

Sleep Data. The nap subjects slept on average 54.69 ± 11.13 minutes (mean ± SD, range = 35 minutes to 69 minutes), with 8.38 ± 4.20 minutes of stage 1 (S1), 31.04 ± 12.14 minutes of stage 2 sleep (S2), and 15.35 ± 13.30 minutes of slow-wave sleep (SWS = stage 3 + stage 4). Of the total sleep time, the nap subjects had 16.00 ± 9.83 percent of S1 (mean ± SD), 56.89 ± 18.71 percent of S2, 27.33 ± 22.81 percent of SWS.

Direct Associative (Face-Object) Memory. Immediately after learning, the nap group (n = 14) recalled 26.21 ± 1.19 (mean ± SEM) AB1 pairs and 25.35 ± 1.49 BC1 pairs, while the no-nap group (n = 17) recalled 27.29 ± 0.63 AB1 pairs and 27.59 ± 0.55 BC1 pairs. Averaging correct responses from the two sets of picture pair, the nap group recalled 25.79 ± 1.24 pairs, while the no-nap group recalled 27.44 ± 0.47 pairs. After the intervening period (nap/no-nap), the nap group recalled 23.79 ± 1.45 AB2 pairs and 23.43 ± 1.44 BC2 pairs, while the no-nap group recalled 23.76 ± 1.04 AB2 pairs and 23.24 ± 1.27 BC2 pairs. Averaging correct responses from the two sets of picture pair, the nap group recalled 23.61 ± 1.31 pairs, while the no-nap group recalled 23.50 ± 0.84 pairs. Independent samples t tests revealed no group difference in performance on the face-object matching before or after the intervening period ($p > 0.18$ for all comparisons).

A repeated-measures ANOVA was conducted to examine retention of the face-object pairs at across the intervening period. Condition (nap/no-nap) served as the between-subject factor and time of test (learning/testing) served as the within-subject factor. There was a significant overall main effect of time ($p < 0.001$). On average, performance on the face-object pairs in the nap group deteriorated by 2.18 ± 0.64 (mean ± SEM) correct responses (8.73 ± 2.45%) from learning to testing while that of the no-nap group deteriorated by 3.94 ± 0.58 (mean ± SEM) correct responses (14.36 ± 2.35%) (condition x time of test interaction, $F_{1,29} = 4.15$, $p = 0.05$ partial $\eta^2 = 0.13$).

Relational (Face-Face) Memory. Subjects' performance on the face-face pairs was significantly above chance (Binomial test of the average number correct (>17), relative to chance performance (7.5 items correct), $p < 0.0001$). Because there was no direct measure of relational memory performance at learning, to account for between groups memory variability between nap and no-nap subjects, an analysis of covariance with learning performance on the face-object pairs serving as a covariate was conducted. The average of AB1 and BC1 was used instead of the average of AB2 and BC2 because the performance immediately after learning is the most accurate indicant of general ability to encode the stimuli. Recognizing, as well, that the probing of AC immediately before the AB2 and BC2 measures would have a priming effect on the delayed test of AB and BC face-object pairs, AB2 and BC2 were not used as the covariate. The nap group performed significantly better than the no-nap group on the relational memory task (One-way ANCOVA, $F_{1,29} = 5.15$, $p = 0.03$, partial $\eta^2 = 0.16$; partial η^2 – eta squared – is a measure of effect size), with nap subjects correctly matching 17.93 ± 1.49 relational pairs, and no nap subjects matching 16.65 ± 1.28 pairs (see *figure 1.2*, which displays performance on AC, adjusted for average learning performance on AB1 and AB2, across the two groups).

Performance on the AC task strongly correlated with the average performance on the AB and BC tasks immediately after learning ($r = 0.67$, $p < 0.001$) and at delayed testing ($r = 0.79$, $p < 0.001$; again, a possible priming effect of AC on AB2 and BC2 should be noted). However, it did not correlate with retention – difference in correct responses immediately after learning and at delayed testing - of neither the AB nor BC list (both p-values > 0.05). Of interest, relational memory of the nap group showed no interdependence with retention of the face-object lists (both p values > 0.40), contradictory to the no-nap group, which showed marginal interdependence between these variables ($p = 0.06$ and 0.10, respectively).

Sleep Stages and Relational Memory. Relational memory performance significantly correlated with total duration of NREM sleep (Stages 2, 3, & 4; $r = 0.62$, $p = 0.02$). To further delineate the unique contribution of individual sleep stages to relational memory, a linear-model regression with correct face-face associations as the criterion and duration of S1, S2 and SWS as predictors was conducted. The analysis assumed simultaneous entry of both predictors. Residuals were normally distributed (One-Sample Kolmogorov-Smirnov Test, $p = 0.86$). The assumption of homoscedasticity was also met as all correlation coefficients between duration of the two sleep stages and the standardized residuals were non-significant ($p > 0.99$). While the overall regression statistic did not reach statistical significance ($R^2 = 0.44$; $p =0.14$), duration of S1, S2 and SWS accounted for 44% of the variance. Most significantly, relational memory showed a significant positive correlation with the duration of SWS (partial correlation, $pr = 0.65$, $p = 0.03$, *figure 1.3a*). S2 and relational memory were marginally but not significantly correlated ($pr = 0.53$, $p = 0.09$, *figure 1.3b*). S1 and relational memory were not correlated ($pr = 0.24$, $p = 0.47$). Although S3 and S4 are conventionally analyzed in tandem as SWS, an additional regression analysis was performed, separating the two stages into individual predictors to discern differential contribution of each, if any. Neither stage yielded a significant partial correlation with relational memory ($pr = 0.59$, $p = 0.07$; $pr = 0.53$, $p = 0.11$, respectively). This analysis, however, might be limited by the lack or small amounts of S4 in more than half of the nap subjects (six nap subjects did not obtain S4, two had less than three minutes of S4). Results yielded when SWS was a single predictor seemed more meaningful.

Because relational memory showed strong correlations with the average performance on the AB and BC tasks immediately after learning and at delayed testing, it is important to discern whether the apparent SWS contribution might have been entangled with these variables. The regression analysis was repeated twice, with the average performance on the face-object tasks immediately after learning and at delayed testing included as a predictor, respectively. Each time, SWS maintained an unequivocal significant positive relationship with relational memory (pr = 0.65, p = 0.04; pr = 0.78, p = 0.008).

Juxtaposed against this clear relationship between NREM sleep, particularly SWS, and relational memory, sleep stage parameters did not correlate with performance on the face-object pairs at learning, at the testing session, nor with the difference in retention of the face-object pairs from learning to testing (all p values > 0.20).

Discussion

The present study investigated the role that sleep plays in memory processing by looking at a fundamental aspect of declarative memory – the establishment of relational links between separately learned memory traces. This process requires the active reorganization and integration of memory traces into a more functionally efficient network, with the shared elements of experiences serving as the binding nodes in the network (Eichenbaum, 2004). Here, subjects who napped exhibited better direct associative memory (i.e. face-object associations – AB, BC pairs) and more importantly, relational memory (i.e. face-face associations – AC pairs), than their counterparts that did not have the advantage of a nap. These results cannot be attributed to circadian factors because subjects in both groups learned and were tested at the same time. The results could neither be accounted for by between-group differences in sleepiness nor intelligence. The most parsimonious explanation is that the associations between these indirectly related face-face pairs were established during the offline period through a sleep-associated process that goes beyond the simple strengthening of the original face-object associations alone. Overall, the results suggest that sleep-specific processes actively promote these two types of declarative memory formation.

At first glance, the direct associative memory (i.e. face-object pairs) findings would appear at odds with previous studies (Plihal & Born, 1997; Tucker et al., 2006) in that napping would appear to prevent memory of the premise pairs from decaying, instead of improving memory. However, one critical difference between the previous studies and the present one is that subjects were purposely exposed to each of the AB and BC lists twice to obtain a high level of retention. The objective here was to test whether relational memory could be built on a well-learned foundation employing the associative premise pairs. In fact, immediately after learning, some subjects reached the maximum score and subjects on average correctly matched 89% of the premise pairs. In sum, subjects had, for the most part, reached a level of maximum performance at learning. Therefore, the current findings are not in contradiction with previous studies, but may serve to lend support to the hypothesis of a stabilizing sleep effect on memory processing (Fowler et al., 1973; Walker, 2005; Talamini et al., 2008). On the other hand, while both are associative tasks, associating pictures of faces and objects may engage different systems, such as the fusiform face area, than associating word pairs used in the aforementioned studies. It remains a possibility that the current findings are task-specific.

More importantly, these findings support that the mnemonic effect of sleep does not end after simply strengthening what is directly learned (i.e., the face-object pairs). Rather sleep appears to also promote a reorganization of memory networks in order to establish common links across disparate items in memory that were not directly learned (e.g., the relational links formed between the faces). The result of this reorganization is a more flexible form of memory at later retrieval. It is not difficult to see how the ability to link separate elements in this adaptive manner would confer a significant evolutionary advantage for an organism. Clearly, relational memory is built upon direct associative memory and thus depends on adequate encoding of directly learned materials. This is reflected in correlations between performance on the face-face task and that on the face-object tasks. However, relational memory does not appear to be as dependent on the retention of direct associative stimuli, suggesting that the emergence of independent memory traces after post-learning integration. The nap group in particular showed no correlation between the two constructs, contradictory to the trend observed in the no-nap group. All things considered, direct associative memory cannot fully account for relational memory. Sleep-dependent processes are, at least, another contributing factor.

As predicted, the duration of SWS obtained during the nap was positively correlated with relational memory, measured by the number of correctly matched relational pairs. The amount of stage 2 sleep and relational memory showed a positive trend but the relationship was not statistically significant. Thus, NREM sleep appears to contribute to the establishment of relational memory with SWS playing the more important role. As individual predictors, stage 3 and stage 4 sleep did not yield significant partial correlations with relational memory, although there was clearly a trend of positive correlation for stage 3 sleep at least. This can be explained by insufficient power in the analysis due to the limited amount of SWS, especially stage 4 sleep, obtained in the nap. At the same time, the results suggest that certain features common to both stage 3 and stage 4 sleep, such as delta oscillatory activities and depressed acetylcholine level, may be important to relational memory processes.

While the mechanisms underlying the effect of SWS on declarative memory processes, and relational memory in particular, have yet to be completely delineated, compelling hypotheses suggest that SWS should specifically confer the greatest advantage for the processing of hippocampus-dependent memories (Buszaki, 1998; Hasselmo, 1999). These hypotheses posit that post-learning SWS produces a systems-level hippocampal-neocortical dynamic, on both the neurophysiological and neurochemical level, necessary to promote the formation of long-term memory representations. There is substantial evidence that SWS may be responsible for the modification of hippocampal and/or neocortical connectivity, hypothesized to be critical to declarative memory consolidation (McClelland, McNaughton & O'Reilly, 1995; O'Reilly & Rudy, 2001). A number of studies have demonstrated that spatiotemporal firing patterns in ensembles of hippocampal place cells exposed to a novel environment during wakefulness show highly correlated activity during subsequent SWS in the hippocampus (Wilson & McNaughton, 1994; Lee & Wilson, 2002) and neocortex (Qin et al., 1997; Ji &Wilson, 2007). These animal studies are nicely complemented by human imaging studies demonstrating the link SWS-specific brain activity and the reorganization/strengthening of memory representations (Peigneux, 2004; Takashima et al., 2006). Additionally, the low acetylcholine level, which is at the nadir during SWS episodes, is known to facilitate memory consolidation (Gais & Born 2004; Hasselmo & McGaughy 2004). Therefore, it appears that SWS may provide the optimal brain state, both neurophysiologically and neurochemically, for the consolidation of declarative memories and for the organization of an efficient network of relational associations. As expected, the hippocampus, which is functionally operative, especially during SWS, has already been shown to play a crucial role in relating disparate memory elements in human (Preston et al., 2004) and animal (Bunsey & Eichenbaum, 1996; Dusek & Eichenbaum, 1997) studies.

While SWS emerges as the strongest predictor of relational memory performance, the involvement of stage 2 sleep is also a possibility. However, there was no significant correlation between stage 2 sleep and relational memory. This is an interesting outcome considering that spindle activity, characteristic of stage 2 sleep, has also been linked to encoding and retrieving of declarative memory (Schabus, et al , 2004; Gais et al. 2002), and is considered another candidate that may underlie the beneficial effect of sleep on declarative memory. Thus, an important question for future research is whether relational memory is selectively dependent on slow wave activity.

It should be noted that while the present study provides direct evidence for sleep-related binding of disparate elements of learned experience, the current findings regarding relational memory may be task-specific because of the use of facial stimuli. However, it is likely that the effect of sleep on relational memory can be generalized because a few recent studies corroborate our findings. One study by Ellenbogen, et al. (2007) demonstrated that when subjects are trained on a hierarchical inferential learning task (A<B<C<D<E), after a night of sleep they are better able to draw inferences between items not learned together during training, and that this is especially the case for second-order associations (e.g., B<E). Our results lend support to this finding and suggest a potential brain state by which this type of processing might optimally occur, namely during SWS. Moreover, sleep may even be important for insight formation, which requires the formation of novel associations that were not directly learned prior to sleep. This was demonstrated in a now classic study by Wagner et al. (2004) who demonstrated that far more subjects who had a night of sleep, compared to a day of wake, gained a valuable insight about a shortcut for solving a simple rule-based number reduction task, although none were consciously aware of this shortcut during training on the task. Another recent study examining false word recall based on common themes (Payne et al., 2009) suggested a similar sleep effect on relational memory of verbal materials. While studies such as these lead us toward new and creative modes of scientific exploration, it is clear that there is still much work to be done to fully understand how sleep facilitates the adaptive use of information.

For example, one important question is whether the sleep-dependent relational memories engage the explicit memory or the implicit memory system. While there is evidence of a sleep effect for both declarative and non-declarative memory tasks (Plihal & Born, 1997; Walker et al., 2003; Gais & Born, 2004; Tucker et al., 2006), it has also been reported that even a non-declarative task requires explicit encoding to benefit from post-learning sleep (Robertson et al., 2004). The matter becomes more complicated considering most, if not all, non-declarative tasks have a declarative component, and perhaps vice versa. It is unclear whether subjects in the current study encoded the face-face associations explicitly. Subjects reported on a post-study questionnaire to be truly surprised by the AC task and to have no active rehearsal of the face-face associations. At the same time, many were aware of the commonality of the object photographs to the two groups of faces after the learning session. Subjects reported a mixture of explicit recall and guessing when performing the AC task. Unfortunately, the reports were not sufficient enough to be correlated with general task performance or to answer to individual questions. Previous studies reported that sleep-facilitated inferential judgment was not aligned with high level of confidence (Ellenbogen et al.,2007) and sleep could even enable the gain of explicit knowledge from implicit encoding (Wagner et al., 2004). Collectively, it seems sleep-dependent relational memory can be either explicit or implicit. This, apparently, is a contradiction to Robertson et al. (2004), which may again be due to differences in task demands, and is certainly a worthwhile direction for future research efforts.

Study II – The effects of immediate vs. delayed post-learning napping on abstraction of relational concepts

Introduction

A growing body of evidence suggests that the state of sleep, even when it is as brief as a ninety minute nap, hosts processes important to different forms of memory (For example: Plihal & Born, 1997; Mednick et al., 2003; Walker et al., 2003), including relational memory (Wagner et al., 2004; Ellenbogen et al., 2007; Payne et al., 2009; Study I of the present research). Relational memory arises from discretely acquired memory traces, integrated into a network through commonalities, thus allowing flexible applications of past experience to a novel but relevant problem (Eichenbaum, 2004). This flexibility of relational memory parallels an important aspect of learning – generalization.

Generalization was first systematically characterized in studies of classical conditioning (Grandine & Harlow, 1948; Razran, 1949). In that context, generalization is the expression of a conditioned response to a stimulus that is new but similar to the conditioned one. While it has since been studied extensively in other simple forms of learning, such as motor learning and perceptual learning (Poggio & Bizzi, 2004; Fahle, 2005; Urcelay & Miller, 2010), generalization in higher cognitive functions, such as the abstraction of a general semantic concept based on specific exemplars, is not as well understood. Given that the abstraction of generality also critically depends on the integration of fragmentary information, it may be a product of relational memory processing. Hence, one focal point of the present study is the question of whether the benefits of daytime napping on relational memory extend to the abstraction of general concepts.

Only a handful of studies have examined the effect of sleep on generalization. One study showed that, after sleep, the extinction of fearful memory generalized from an extinguished conditioned stimulus to a similarly conditioned but unextinguished one (Pace-Schott et al., 2009). The facilitating effect of sleep is not limited to simple forms of memory. A second study found that infants who napped were better able to generalize an abstract rule in an artificial language to stimuli that were similar but not identical to those learned in a pre-nap familiarization procedure (Gomez et al., 2006). However, this finding, while being interesting, did not establish a causal link between napping and generalization because this study employed a non-experimental procedure.

The present study represents the first experimental investigation of the relationship between daytime napping and the abstraction of general concepts. Subjects learned English meanings of Chinese characters that shared ideographical components called radicals, each of which represented a general semantic concept. Characters sharing the same radical had related meanings. In order to assess the abstraction of the general concepts, subjects were later asked to match unstudied characters, with previously seen radicals, to their English meanings (multiple choice task) as well as to explicitly state the concepts represented by the radicals (radical task). Subjects who napped were hypothesized to have better relational memory, namely matching more unstudied Chinese characters to their English meanings and correctly stating the concepts of more radicals.

Another focal point of the present study is the temporal separation between learning and the post-learning nap. The present study compared subjects whose intervening period (nap or no-nap) immediately followed learning to subjects whose intervening period occurred after a delay. The delay approximated the duration of the intervening period for the immediate groups. The purpose of this manipulation was two-fold. First, it examined a long-standing debate about whether the beneficial effect of sleep on memory is based on active mechanisms or simply due to a reduction of external sensory stimulation (Jenkins & Dallenbach, 1924; Vertes & Siegel, 2005). If the latter were true, the delayed nap group with the same amount of waking experience as the immediate wake group would show no benefit from napping. Otherwise, both the immediate and delayed nap groups would show enhanced relational memory.

Second, it allowed probing of another important question – how soon after learning does sleep need to occur in order to benefit relational memory? On one hand, sleep may simply be a state that optimizes eventual memory processes following acquisition. In this case, the effect of sleep would be the greatest if it immediately follows learning and would fade with increasing delay. On the other hand, there may be active mechanisms during sleep, aiding memory processing regardless of the time of sleep onset. The majority of previous research used paradigms in which sleep occurred immediately after learning. Only recently has the possible memory effect of delayed sleep been suggested (Backhaus et al., 2007; and an ongoing study in our laboratory) although the sample of the Backhaus et al. study was limited to children from 9 to 12 years-old. Using both immediate and delayed groups, the present study elucidated whether the relationship between daytime napping and relational memory is dependent on their temporal proximity.

On a final note, the majority of existing knowledge supports the notion of differential roles of individual sleep stages in memory processing (For reviews, see: Buszaki, 1998; Hasselmo, 1999; Tononi & Cirelli, 2002, 2006). Slow-wave sleep (SWS) seems to particularly favor declarative memory (Yaroush et al., 1971; Plihal & Born, 1997; Tucker et al., 2006) while rapid eye movement (REM) sleep preferentially favors procedural memory (Plihal & Born, 1997; Walker et al., 2002, 2003). The present study used regression analysis to delineate the contribution of each sleep stage to abstraction of generality. Keeping harmony with previous research and given that declarative materials were used in the current tasks, SWS was expected to be the foremost contributor.

Methods

Subjects

Fifty-eight subjects were originally recruited from the undergraduate population of the City College of New York. They were in good health and free of all medications that might either impair or facilitate sleep. Each subject kept a sleep log for a week prior to the study. Any subject having an irregular sleep schedule was excluded from the study. In addition, any subject who was not a native English speaker or who had previous experience with Chinese characters or Japanese Kanji was excluded from the study due to the nature of the task. Subjects were asked to refrain from drinking coffee or alcohol during the 24 hours preceding participation. All subjects had normal or corrected to normal vision. Out of the original fifty-eight subjects, seven were excluded from data analysis for the following reasons. One subject from the immediate no-nap group did not comply with instructions of refraining from caffeine. One subject from the immediate nap group and three subjects from the delayed nap group had excessively fragmented sleep. One subject from the delayed nap group fell asleep during the delay. One subject from the delayed no-nap group admitted to having previous knowledge of Japanese Kanji after completing the tasks. The remaining fifty-one subjects (35 females) were on average 18.98 ± 1.75 (mean ± SD) years of age. There were 15 subjects (11 females) in the immediate nap group, 14 subjects (9 females) in the immediate no-nap group, 11 subjects (7 females) in the delayed nap group, and

11 subjects (8 females) in the delayed no-nap group. All subjects signed an informed consent form. The study was approved by the City College of New York Institutional Review Board.

Tasks

Subjects learned English meanings of twenty-one Chinese ideographs called characters, consisting of seven groups. The three characters within each group shared the same left component called a radical (see *figure 2.1a*). Each radical represented a general concept and characters sharing the same radical had related meanings. Immediately after learning, subjects were given a cued recall task in which they attempted to type the English meaning of a given Chinese character. There were a total of fourteen characters in the cued recall task.

After either a daytime nap or a period of wakefulness, subjects first matched the English meaning to a given character in a multiple-choice task (see *figure 2.1b*). Importantly, this task consisted of a mixture of seven old characters and twenty-one new characters, which the subjects had not seen before but shared the same seven radicals as the old characters previously seen. There were four possible answer choices for each given character, one of which was the correct English meaning and the other three distracters. The answer choices were selected so that if the subjects had extracted the general concept of the radical, they would be able to deduce the correct answer even if they had not been exposed to that given character before. Therefore, there were two components in this task – associative recognition memory of English meanings of old characters and expression of relational memory in the new characters.

Lastly, subjects were shown the seven radicals in isolation and were asked to explicitly type the meaning of the isolated radicals (radical task, see *figure 2.1b*). For a given radical, subjects were given three points if they typed the correct meaning in the space provided next to this radical. They were given two points if the correct meaning of this radical was among the answers but mismatched to a different radical. They were given one point if they typed the meaning of a specific character that contains the radical instead of the general concept of the radical itself. This task aimed to assess the extraction of a general concept. Therefore, scores for different types of responses were ranked to reflect this priority.

All of the presentation of stimuli and tasks were presented on a 50cm monitor screen in the format of Microsoft PowerPoint slides. All words were presented in 40-point font.

Procedures

Subjects arrived at 10:30am at the Laboratory of Cognitive Neuroscience and Sleep at The City College of New York. Upon arrival, subjects were asked to sign a consent form explaining the nature of the research, complete a demographics questionnaire and the Epworth Sleepiness Scale (Johns, 1991), which is a measure of general daytime sleepiness that assesses subjects' tendency to fall asleep in various situations. Subjects were then shown around the laboratory to familiarize them with the laboratory environment and the sound-attenuated bedrooms. The subjects then completed a subset of the Multidimensional Aptitude Battery-II (MAB-II; Jackson, 1998). The MAB-II is a standardized paper-and-pencil test commonly used to assess intelligence. The selected subtest was vocabulary. The vocabulary subset was used to assess subjects' verbal aptitude, which might be a factor in ability to encode the English meaning of the Chinese characters. It also has the highest correlations with intelligence measured by the Wechsler Adult Intelligence Scale**.** The subtest took 7 minutes to complete.

At approximately 11:00am, electrodes were applied to all subjects to ensure a similar experience for everyone. At approximately 11:30am, subjects learned English meanings of the

Chinese characters, presented on a 50-cm flat-panel screen (approximately 0.6 m from the subjects) using Microsoft PowerPoint slideshow. Sitting in a comfortable chair, they viewed the Chinese characters and their corresponding English translations. All words were presented in 40 point font. Each character and its English translation were displayed for 2 seconds with a one-second inter-stimulus interval. Subjects were instructed to pay close attention to each and to try to memorize its English meaning. A total of twenty-one characters were presented, consisting of seven groups of three characters sharing a common conceptual radical, hence conceptually related. The characters were presented in a pseudo-random order such that characters with the same radical were not closely positioned. Following two run-through exposures to the characters (to ensure subjects had adequately encoded the characters and their meanings) subjects completed the cued recall test, in which fourteen of the learned characters were given as cues and the subjects were to input its meaning with a keyboard. Subjects were allowed as much time as they needed to complete the task. The response time was recorded. The entire learning phase took about 30 minutes to complete.

After completing the learning phase, subjects were randomly assigned to one of four groups – the immediate nap group, the immediate no-nap group, the delayed nap group, or the delayed no-nap group. For a summary of the protocol, see *figure 2.2*.

At approximately12:00pm, the immediate nap group subjects were taken to a sound-attenuated bedroom to attempt to take a nap. Allowed to get comfortable in the bed, the electrode cable was connected to the polygraph input box. The immediate no-nap subjects were also taken to a second sleep chamber, and were asked to sit in a semi-recumbent position with the electrode cable connected to the polygraph input box. The immediate no-nap subjects watched videos about ocean life while the immediate nap group slept. When the nap subjects were awakened, subjects from both groups exited the chambers and electrodes were removed.

Nap subjects were permitted an approximately 90-minute opportunity to attempt to sleep. If subjects obtained SWS or REM sleep during this time they were allowed to sleep until it appeared the SWS or REM sleep period was coming to an end. Subjects were awakened from sleep stages 1 or 2 when possible. Therefore the 90-minute window to nap was slightly expanded for some subjects to accommodate the procedure of waking up subjects.

All subjects were monitored online when they were in the sleep chambers by a digital EEG acquisition software (Gamma System-Grass/Telefactor^{tm}) using a five-channel polysomnographic montage, which included electroencephalography (EEG recorded between electrode pairs C3-A2 and C4-A1), electro-oculography (EOG), and chin-electromyography (EMG) channels. The sleep recordings were scored using the international criteria of Rechtschaffen and Kales (1968).

After exiting the sleep chambers subjects were led to a quiet sitting room in the laboratory where electrodes were removed. Half an hour after exiting the sleep chambers, at about 2:00pm for the immediate nap group and the immediate no-nap group, subjects were asked to match the correct English meaning to given Chinese characters in the multiple-choice task, which consisted of a mixture of old and new characters. Upon completion, they were asked to state explicitly the meaning of isolated radicals (radical task). In all tasks, subjects were instructed to guess even if they were unsure of the answer. Subjects were allowed as much time as needed for all three tests. Response time was recorded.

At the end of the study, subjects were given five-point Likert scales to assess their motivation in performing the tasks. The average rating was calculated for analyses. Subjects were also asked to rate the difficulty of each task and their interest in the entire study on a scale from 1 to 10, one being the minimal level and ten being the maximal level.

Procedures for the delayed groups were similar except that the delayed nap group had a two-hour delay before their post-learning nap. Their window to nap started at about 2:00pm. They were given the multiple-choice task at about 4:00pm. They watched videos about ocean life in the sleep chamber during the delayed period. The delayed no-nap group watched videos for the entire period between the learning phase and the testing phase when they were given the multiple-choice task and the radical task.

Results

Sleepiness and Intelligence Measures. A one-way ANOVA with group membership as the between-subject factor was performed for the sleepiness scores measured by the Epworth Sleepiness Scale. Data from two subjects, one from the immediate nap group and one from the delayed nap group were missing. There was no difference in sleepiness between the four groups ($F_{3,45} = 0.88$, $p = 0.46$).

Intelligence scores measured by the MAB-II vocabulary subtest were standardized. *Z*-scores were calculated and used in the subsequent analyses. A one-way ANOVA with group membership as the between-subject factor was performed. There was no difference in intelligence between the four groups ($F_{3,47} = 1.35$, $p = 0.27$).

For a summary of group means and SEMs for these measures, see *table 2.1*.

Post-Study Subjective Report of Motivation, Task Difficulty, and Interest in Study. Between-group comparisons showed all four groups were comparable in each subjective measure (all p values > 0.35).

Response Time. Subjects' average response time was 4:31 ± 1:42 (min:sec, mean ± SD) for the cued recall task, 6:20 ± 1:50 for the multiple choice task, 2:47 ± 1:11 for the radical task, respectively. Maximum response time was 11 minutes and 6 seconds. No significant difference in response time was found between the groups on any of the tasks (One-way ANOVA, all p values > 0.15, see *table 2.2 for group means and SEMs*).

Sleep Data. The nap subjects took an average of 8.08 ± 5.93 minutes (mean ± SD) to fall asleep. Their average sleep efficiency (total sleep time divided by total time in bed) was 80.71 ± 10.82 percent. They slept on average 68.79 ± 13.56 minutes (range = 46 minutes to 101 minutes), with 5.88 ± 4.36 minutes of stage 1 (S1), 33.50 ± 12.21 minutes of stage 2 sleep (S2), 6.25 ± 4.38 minutes of stage 3 sleep (S3), 14.44 ± 13.95 minutes of stage 4 sleep (S4), 20.69 ± 14.95 minutes of slow-wave sleep (SWS = stage 3 + stage 4), 8.42 ± 10.25 minutes of rapid eye-movement sleep (REM). Of the total sleep time, the nap subjects had 9.29 ± 7.81 percent of S1, 49.00 ± 16.73 percent of S2, 9.05 ± 6.53 percent of S3, 20.77 ± 20.43 percent of S4, 29.81 ± 21.38 percent of SWS, 11.44 ± 13.59 percent of REM.

The delayed nap subjects had significantly longer total sleep time (77.45 ± 3.81 minutes, mean ± SEM) compared to the immediate nap subjects (63.17 ± 2.93 minutes). This may be due to the delayed nap subjects being more likely to reach SWS and REM than the immediate nap subjects (72% of the delayed nap subjects compared to 53% of the immediate nap subjects) although the difference did not reach statistical significance (Chi-square, p = 0.18). Hence, a bigger proportion of the delayed nap subjects were allowed to nap longer and cycle out of SWS or REM before they were woken up. No difference was found in any other sleep parameter.

For a summary of sleep parameters across groups, see *table 2.3*.

Cued Recall Task. Performance on the cued recalled task at learning was measured by the number of correct English meanings recalled. Average performance ± SEM for each group was, respectively, as followed: Immediate Nap = 8.53 ± 0.85; Immediate No-nap = 8.36 ± 0.75; Delayed Nap = 8.00 ± 1.00; Delayed No-nap = 8.26 ± 0.41. There was no group difference in recalling English meanings of individual characters in the learning phase (One-way ANOVA, $F_{3,47} = 0.09$, $p = 0.96$). Post hoc tests confirmed the four groups were comparable (LSD, all p values > 0.66).

Multiple Choice Task. Performance on the old characters and the new characters in this task was analyzed separately as they measured different kinds of memories – direct associative recognition memory and relational memory, respectively. Number of correct answers was used as a measure of memory in each case. A between-subject 2x2 ANOVA, where the nap condition (nap / no-nap) and the time condition (immediate / delayed) serve as factors, was conducted for performance on the old characters. Performance on the cued recall task at the learning phase was used as a covariate to control for individual variability in learning Chinese characters. There were no significant main effects for either the nap condition ($F_{1,47} = 0.92$, $p = 0.34$) or the time condition ($F_{1,47} = 0.0001$, $p = 0.99$). There was a significant interaction between nap and time conditions ($F_{1,47} = 5.50$, $p = 0.02$, $\eta^2 = 0.11$, see *figure 2.3*). An ANCOVA using cued recall performance as the covariate found no difference between the immediate nap (mean ± SEM: 5.00 ± 0.35) and the immediate no-nap (4.50 ± 0.42) group ($F_{1,27} = 0.90$, $p = 0.35$). On the hand, a similar analysis revealed a significant difference between the delayed nap (4.09 ± 0.44) and the delayed no-nap (5.18 ± 0.35) group ($F_{1,20} = 7.63$, $p = 0.01$). Particularly, the delayed no-nap subjects recognized more English meanings for the old characters than the delayed nap subjects, when performance on cued recall at learning was considered.

A similar 2x2 ANOVA was conducted for performance on the new characters. There was a marginally significant main effect for the nap condition ($F_{1,47} = 3.99$, $p = 0.05$, $\eta^2 = 0.08$, see *figure 2.4*) such that the nap subjects, compared to the no-nap subjects, correctly matched English meanings to more Chinese characters they had not previously seen. Average scores for each group were as follows (mean number of correct responses ± SEM): immediate nap 8.27 ± 0.68, immediate no-nap, 6.64 ± 0.50, delayed nap 8.45 ± 1.23, delayed no-nap 6.91 ± 0.69. There was no significant main effect for the time condition ($F_{1,47} = 0.10$, $p = 0.76$) or significant interaction between nap condition and time condition ($F_{1,47} = 0.002$, $p = 0.97$).

Radical Task. A between-subject 2x2 ANOVA, where the nap condition (nap / no-nap) and the time condition (immediate / delayed) serve as factors, was conducted for performance on the radical task. Similar to analyses for the multiple-choice task, performance on the cued recall task at the learning phase was used as a covariate. There was a significant main effect for the nap condition ($F_{1,47} = 6.69$, $p = 0.01$, $\eta^2 = 0.13$, see *figure 2.5*). Specifically, the nap subjects were better able to explicitly state the general concept of isolated radicals than their no-nap counterparts. Average scores for each group were as follows (mean score on the radical task ±

SEM): immediate nap 7.60 ± 0.88, immediate no-nap, 4.50 ± 0.71, delayed nap 7.91 ± 1.28, delayed no-nap 5.82 ± 1.29. There was no significant main effect for the time condition ($F_{1,47}$ = 0.91, p = 0.35) or significant interaction between nap condition and time condition ($F_{1,47}$ = 0.22, p = 0.64).

Closer examination of the nap effect within each time condition showed the immediate nap group (mean ± SEM: 7.60 ± 0.88) having significantly better performance (p = 0.01) on the radical task than the immediate no-nap group (4.50 ± 0.71). Although the performance of the delayed nap group (7.91 ± 1.28) was numerically better than the delayed no-nap group (5.82 ± 1.28), the difference did not reach significance. The variance was noticeably, although not significantly, greater in the delayed groups.

Time Effect. The nap groups and the no-nap groups were analyzed separately to further examine the time effect on performance. The delayed no-nap subjects had marginally better recognition memory of the old characters than the immediate no-nap subjects (mean ± SEM: 5.18 ± 0.35 vs. 4.50 ± 0.42.correct responses; $F_{1,23}$ = 3.07, p = 0.09). Comparing the immediate condition and the delayed condition revealed the no significant time effect on any other tasks (all p values > 0.15) in neither the nap nor no-nap subjects.

Amount of External Interference. The possible explanation of nap effect by reducing external interference is addressed by comparing the delayed nap group to the immediate no-nap group because the two had the same amount of wakefulness between learning and testing. ANCOVAs using cued recall performance as the covariate showed the delayed nap subjects to be superior in stating concepts represented by isolated radicals (delayed nap: 7.91 ± 1.28 [mean ± SEM] vs. immediate no-nap group: 4.50 ± 0.71; $F_{1,23} = 8.20$, $p = 0.009$, $\eta^2 = 0.27$). They also had more correct responses to the new characters but the difference did not reach significance (delayed nap: 8.45 ± 1.23 vs. immediate no-nap group: 6.64 ± 0.50; $F_{1,23} = 2.22$, $p = 0.15$). The lack of significance can be explained by the significantly greater amount of error variance in the delayed nap group (Levene's Test of Equality of Error Variances, $p = 0.04$). The two groups did not differ in recognizing the old characters ($F_{1,23} = 0.48$, $p = 0.49$).

Sleep Stages and Memory. To delineate whether there is unique contribution of individual sleep stages to memory, linear-model regression analyses were conducted; respectively, for associative recognition memory measured by performance on the old characters in the multiple-choice task, and relational memory measured by performance on the new characters in the multiple-choice task and the radical task. The duration of S1, S2, SWS and REM served as predictors. The analyses assumed simultaneous entry of all predictors. Residuals in all three analyses were normally distributed (One-Sample Kolmogorov-Smirnov Test, all p values > 0.15). The assumption of homoscedasticity was also met as all correlation between duration of the individual sleep stages and the standardized residuals were non-significant ($p > 0.99$). No significant partial correlation between the duration of individual sleep stages and task performance was detected (all p values > 0.10).

Discussion

The role of sleep in a flexible and adaptive form of memory called relational memory is one of the most important questions in this field of research. Taking advantage of the ideographical nature of Chinese characters, the present study shows a beneficial effect of daytime napping on extracting a general concept from disparately learned but semantically related stimuli. While associative recognition memory of the learned Chinese characters was comparable when subjects attempted to match the characters to their English meanings, the nap subjects showed better relational memory as measured by the new characters. Moreover, the nap subjects performed better when asked to explicitly state the concepts represented by isolated radicals. The results are consistent with the notion of sleep having a role in processes that integrate and reorganize memory traces. General learning ability, daytime sleepiness, intelligence, task difficulty or subjects' motivation and interest in the tasks cannot explain the effect of napping on relational memory. The possible involvement of circadian factors is also eliminated because each nap group was accompanied by a no-nap control group that learned and was tested at the same time of the day.

Napping facilitates relational memory regardless of whether it occurs immediately after learning or after a delay. The findings corroborated the handful of studies consisting of this temporal element (Walker et al., 2002; Backhaus et al., 2007). Of equal importance, comparison between the delayed nap group and the immediate no-nap group showed the beneficial effect of daytime napping on relational memory, and is not a result of reduction in external sensory stimulation. The findings suggest an active, rather than passive, role of sleep in memory processing. The underlying mechanisms may involve neurophysiological, or neurochemical activities, or both. The activities may occur both on the system level such as global neural synchronization and desynchronization (Tononi & Cirelli, 2002, 2006), temporally correlated hippocampal and cortical activities (Buszaki, 1998), and sleep-stage-dependent change in acetylcholine level (Hasselmo, 1999), as well as on the cellular level such as neural reactivation

(Wilson & McNaughton, 1994; Lee & Wilson, 2002) and experience-dependent gene expression (Ribeiro et al., 1999).

The present study failed to detect any significant correlation between duration of individual sleep stages and performance. This is unexpected given the most widely-accepted sleep and memory models are stage-specific (Buszaki, 1998; Hasselmo, 1999; Tononi & Cirelli, 2002, 2006) and a positive correlation between SWS and performance on a different relational memory task (Study I of the present research). However, a closer examination of theoretical models, data and tasks makes the presumption of such correlation less certain. Alternatively, there are several models that posits the sleep effect on memory to the inherent architecture of sleep, and the cycling of sleep stages rather than any sleep stage per se (Guiditta et al., 1995; Smith, 2001; Pennartz et al., 2002; Walker, 2005).

Tasks in both Study I and the present study have a relational memory quality, both examining links not directly learned. However, the task used in Study I centers on the transitivity of direct associative memory, whereas tasks in the present study call for abstraction of general concepts. The present tasks, being more abstract, are likely to require more complex neural processes. In fact, Payne et al. (2009) using the DRM task (In the Deese–Roediger–McDermott after studying a list of semantically related words, subjects often falsely recall an associated word being in the list, even though this word has not been actually presented.), which is more similar to the present tasks, similarly reported no correlation between any sleep parameter and the relational aspect of the task. Taken together, abstraction of generality may be a function too complex to depend solely on any specific sleep stage.

A striking difference between the present study and the majority of existing literature is the observation of the delayed nap group recognizing fewer old Chinese characters than the delayed no-nap group. It should be noted that there were only seven old characters used to assess associative recognition memory. This may render the analysis less meaningful. On the other hand, because the memory tasks were meant to be a surprise, subjects were not explicitly instructed to avoid rehearsal of the learned materials. Unlike the majority of previous studies, subjects were not allowed to leave the laboratory or engage in activities of their choice during the intervening retention period. They watched non-arousing videos of ocean life while they were awake. It is possible that the delayed no-nap subjects rehearsed the old characters during the intervening period. They had the largest window for rehearsal, if that did occur. They did recognize the largest number of tested old characters out of the four groups although the difference is small and was not statistically significant when compared to the immediate nap and no-nap subjects. With that said, it is equally possible that the processing of relational memory could interfere with the processing of the direct associative memory. A hallmark of the evolution of episodic memory is the retention of gist and eventual fading of specific details tied to the original experience. Sleep may accelerate this morphology of memory.

Our results make clear that sleep is important for the abstraction of generality. This is evident with even a brief period of daytime napping. Findings in the present study show the necessity of further research regarding the role of sleep in relational memory. It is important to identify the sleep-dependent mechanisms that facilitate relational memory to discern the effect of sleep on different relational memory tasks, and to delineate possible interaction between processing of relational memory and processing of memory that gives rise to relational links.

Study III – The immediate and long-term effects of daytime napping on context-dependent relational memory

Introduction

Episodic memory, declarative memory of autobiographical experience (Tulving, 1973), is formed by conjunctively associating items (e.g. what, who) in a particular autobiographical event. The importance of contextual cues, including temporal and spatial information (i.e. when and where), of the event for this intra-episode or direct association is well-documented (For reviews, see Eichenbaum, 2004; Smith & Mizumori, 2006). In contrast, the role of temporal and spatial context in relational memory – the indirect association of items across separate episodes based on commonalities – remains unclear. Theoretically, relational memory can be formed across episodes with overlapping temporal and spatial context.

In the field of sleep and memory, the link between sleep and episodic memory has long been established (For example: Yaroush et al., 1971; Fowler et al., 1973; Plihal & Born, 1997, 1999; Gais & Born, 2004; Tucker et al, 2006), while evidence is emerging for a facilitating effect of sleep on relational memory (Wagner et al., 2004; Ellenbogen, 2007; Payne et al., 2009; Study I & II of the present research). Thus far, sleep studies on episodic memory have overwhelmingly focused on direct associative memory (For example: Plihal & Born, 1997, 1999; Gais & Born, 2004; Tucker et al, 2006) with some studies examining spatial memory (Talamini et al., 2008; Wamsley et al., 2010; Peigneux et al., 2004; Rauchs et al., 2008). Rarely have sleep and memory researchers explored the contextual value of both temporal and spatial information (Drosopoulos et al., 2007 examined the influence of the temporal order on recall of word list), let alone the influence of these types of information on relational memory. The present study fills the gaps in existing research by addressing two important questions: Can temporal and spatial contexts serve as the commonalities that give rise to relational memory? And, if so, does sleep, as brief as a daytime nap, mediate the process?

Specifically, subjects encoded different groups of pictures with overlapping temporal and spatial contexts. After a retention period with or without a nap, subjects had to judge group membership of picture pairs, including pairs that did not belong to the same group but had shared either the same temporal context or spatial context. The influence of contextual cues on relational memory is operationalized as false alarm rates on such temporally-related non-match and spatially-related non-match items. Although the false alarm rate is not a conventional measure of memory formation, a handful of recent studies demonstrated the ability of sleep to promote false memory of critical theme-words not presented at encoding but semantically related to the encoded ones (Payne et al., 2009; Darsaud et al., 2010).

At the same time, although scarce, there is evidence for temporal and spatial memory being processed during sleep. Several studies examined the effect of sleep on spatial memory. On the behavioral level, sleep appeared to facilitate performance on a location-object association task (Talamini et al., 2008) and a route-learning task (Wamsley et al., 2010). On the neuronal level, route-learning led to increased activity during sleep in the hippocampus and its surrounding area, which are important to spatial memory (Peigneux et al., 2004). Correspondingly, post-learning sleep shifted task-related neural activity from the parahippocampal region to another subcortical structure, the caudate (Rauchs et al., 2008). There has been one study that investigated the processing of temporal context during sleep (Drosopoulos et al., 2007). In this study, post-learning sleep resulted in superior recall of word triplets only when the order of words at retrieval matched that at encoding, suggesting that sleep aids the integration of temporal context into episodic memory.

In light of all of the available evidence, the nap subjects in the present study were hypothesized to be more likely to falsely associate pictures that belong to different triplets but are indirectly related either through temporal or spatial contexts.

In addition to the two important research questions discussed earlier, the present study tackles a third one – whether sleep has a long-term effect on relational memory. The majority of previous research probed memory either immediately after (For example: Phihal & Born, 1997, 1999; Wagner et al., 2004; Tucker et al., 2006; Ellenbogen et al., 2007; Payne et al., 2009) or shortly after the post-learning sleep (typical in sleep deprivation studies to allow recovery nights of sleep, for example: Stickgold et al., 2000; Ferrara et al., 2006; Gais et al., 2006). This question certainly deserves far more attention because it can elucidate the importance of sleep in memory processing. If sleep is an essential operative stage of memory processing, it should have a long-lasting effect on memory. In contrast, the lack of a persistent effect would suggest that sleep is unlikely to be crucial in memory processing. So far, the few studies that have considered the long-term effect of sleep point to a positive result. Takashima and coworkers (2006) found a correlation between post-learning sleep and systematic changes in neural activity when subjects performed a picture recognition task six months after encoding. In a behavioral study, Wagner and his colleagues (2006) showed that sleep, as brief as three hours, immediately following learning led to preservation of emotional memories for over four years. The present study expanded on these findings by examining both the immediate and long-term (with a one-week delay) effect of a post-learning nap on relational memory. Based on previous findings, a positive long-term effect of napping on relational memory was expected.

Lastly, the majority of existing literature favors the hypothesis that individual sleep stages contribute to memory processes differentially (For reviews, see: Buszaki, 1998; Hasselmo, 1999; Tononi & Cirelli, 2002, 2006). While there is considerable evidence linking slow-wave sleep (SWS) to episodic memory (Plihal & Born, 1997; Tucker et al., 2006; Takashima et al., 2006), the link between individual sleep stages and relational memory is not as clear. Study I of the present research found a positive correlation between SWS and relational memory, whereas Study II, along with a previous study from another laboratory (Payne et al., 2009), found no correlation. It is noteworthy that while all three studies used tasks with the characteristics of relational memory, the stimuli and task in each were quite different. Aiming to clarify the mixed findings, the present study used regression analyses to delineate the link between each sleep stage and false alarm rate on the temporally and spatially related non-match questions.

Methods

Subjects

There were originally thirty-four subjects. Data of four subjects were excluded for the following reasons. Two subjects assigned to the nap group could not fall asleep or maintain sleep. One subject completed the tasks but later admitted to not understanding the instructions. One subject could not reach the criterion, in fact with worsening performance, after three trials of the membership recognition task given immediately after learning. The remaining thirty subjects, with an average age of 20.33 ± 2.37 (mean ± SD), were in good health, free of all medications that might either impair or facilitate sleep. There were 15 subjects in the nap group (10 females, mean age = 20.93 ± 2.71) and 15 subjects in the no-nap group (9 females, mean age = 19.73 ± 1.87). Each subject kept a sleep log for two weeks, one week prior to the study, and another week between study day 1 and study day 2. Any subject having an irregular sleep schedule was excluded from the study. Subjects were asked to refrain from drinking coffee or alcohol during the 24 hours preceding participation. All subjects had normal or corrected to normal vision. All subjects signed an informed consent form. The study was approved by the City College of New York Institutional Review Board.

Tasks

The stimuli were 24 colored abstract pictures randomly grouped as 8 triplets. Subjects were instructed to memorize group membership of the pictures. The stimuli allowed probing of both direct associative and relational memory based on temporal and spatial context. Members of each triplet were presented in a specific and consistent order, and each at a particular quadrant of the monitor. Members in the same triplet did not share the same spatial location. As a result, a picture could be related to ones outside of its triplet group by either sharing the same temporal order (temporally-related – TR, i.e. the number 2 pictures) or the same spatial location on the screen (spatially-related – SR, i.e. pictures located in the upper right quadrant, see *figure 3.1a*). Therefore, the stimuli allowed the establishment of direct associative memory based on group membership and the establishment of relational memory based on temporal and spatial information.

The primary task in this study was membership recognition, in which the subjects judged whether a pair of pictures belonged to the same triplet. Obviously, the pictures could be from the same triplet or they could be unrelated in any way (see *figure 3.1b*). Correct responses on these questions would reflect direct associative memory. Additionally, the pictures could belong to different triplets but be related based on temporal and spatial information. It is crucial to note that false alarms on these questions reflected the establishment of relational links. It is important to note the different types of non-match questions and different types of false alarms – non-related (NR), temporally-related (TR) or spatially-related (SR, see *figure 3.1b*). False alarm rates on the TR and SR questions were indicative of relational memory establishment.

Baseline direct associative memory was assessed by a membership recognition task with only match and non-related non-match questions. Relational memory was assessed by a similar task except all possible categories of questions – match, NR, TR, and SR non-match - were included. To distinguish its aim from the former version, this version of the task will be called the relational memory task.

A separate context recall task_was given whereby subjects explicitly state, of a given picture, the presentation order in its triplet (e.g. number 1, 2 or 3) and location (e.g. one of the four quadrants).

Procedures

The study took place on two separate days, one week apart. On day 1, subjects arrived at 10:30am at the Laboratory of Cognitive Neuroscience and Sleep at The City College of New York. Upon arrival, subjects were asked to sign a consent form explaining the nature of the research, complete a demographics questionnaire, and complete the Epworth Sleepiness Scale (Johns, 1991). The Epworth Sleepiness Scale is a measure of general daytime sleepiness by asking subjects' tendency to fall asleep in various situations. Subjects were then shown around the laboratory to familiarize them with the laboratory environment and the sound-attenuated bedrooms. The subjects then completed two subtests of the Multidimensional Aptitude Battery-II (MAB-II; Jackson, 1998). The MAB-II is a standardized paper-and-pencil test commonly used to assess intelligence. The two selected subtests were vocabulary and picture completion, which are among subtests with highest correlations with intelligence measured by the Wechsler Adult Intelligence Scale**.** Each subtest took 7 minutes to complete.

At about 11:00am, a five-channel polysomnographic montage, which included electroencephalography (EEG recorded between electrode pairs C3-A2 and C4-A1), electro-oculography (EOG), and chin-electromyography (EMG) channels, was applied to all subjects to ensure a similar experience for everyone.

At approximately 12:00pm, subjects were exposed to the stimuli, presented on a 50-cm flat-panel screen (approximately 0.6 m from the subjects) using Microsoft PowerPoint. They were instructed to memorize group membership of the pictures. Immediately after a 2-run exposure, they were trained to a criterion of 70% correct responses on a membership recognition task with only match and non-related non-match questions. They then were asked to explicitly recall the presentation order and location of individual pictures. The tasks took about 30 minutes to complete.

Subjects were then randomly given either an approximately 90-minute opportunity to nap or to stay in a sleep chamber watching a light comedy. The video was chosen to minimize arousal, cognitive activity, and visual interference with the task stimuli. All subjects were monitored online by a digital EEG acquisition software (Gamma System-Grass/Telefactor^{tm}). If nap subjects obtained SWS and/or REM sleep during this time, they were allowed to sleep until it appeared the SWS or REM period was coming to an end. Subjects were awakened from sleep stages 1 or 2 if possible. Therefore the 90-minute window to nap was slightly expanded for some subjects to accommodate the procedure of waking up subjects. The sleep recordings were scored using the international criteria of Rechtschaffen and Kales (1968). At the end of the nap period, the nap and no-nap subjects exited their respective bedrooms and electrodes were removed.

After the intervening period, subjects were asked to perform the membership recognition again, except this time all possible categories of questions were included to examine not only direct associative memory but relational memory as well. This task concluded study day 1. Subjects were instructed to keep a regular sleep schedule and to document their sleep pattern for the following week. Given that sleep was a critical manipulation of the present study, it was very important that subjects maintained a regular sleep schedule and have adequate amount of sleep during the one-week delay. To ensure this, subjects were asked to wear a small wrist band (see *figure 3.2*) when they went to sleep each night during the one-week delay. They were told that it was a device that would record their sleep time and sleep pattern. In fact, the device consisted of only a small power button attached to two wires and a battery in a soft USB case. To familiarize the subjects to the wrist band, they were advised to start wearing it two hours before they went to sleep on the first night of the one-week delay.

After one week, subjects returned to perform the membership recognition task with relational memory questions, followed by explicit recall of presentation order and location of stimuli. Subjects filled out a post-study motivation questionnaire before leaving the laboratory.

The behavioral tasks and the procedure are summarized in *figure 3.1*.

Results

Sleepiness, Motivation, and Intelligence Measures. Independent t tests revealed no group difference in sleepiness, motivation, or intelligence measured by the two MABII subtests (all p values > 0.11). For a summary of group means and SEMs, see *table 3.1*.

Sleep Data. Average latency to sleep onset of the nap subjects was 8.77 ± 5.82 minutes (mean ± SD). Their average sleep efficiency was 80.28 ± 7.21 percent. Mean total sleep time was 69.90 ± 14.03 minutes (range = 37 minutes to 90.50 minutes), with 4.70 ± 3.14 minutes of stage 1 (S1), 28.40 ± 9.61 minutes of Stage 2 sleep (S2), 7.10 ± 3.58minutes of Stage 3 sleep (S3), 15.60 ± 13.09 minutes of Stage 4 sleep (S4), 20.69 ± 14.95 minutes of slow-wave sleep (SWS = stage 3 + stage 4), 7.73 ± 8.26 minutes of rapid eye-movement sleep (REM). Of the total sleep time, the nap subjects had 8.22 ± 6.88 percent of S1, 45.32 ± 12.76 percent of S2, 11.11 ± 5.21percent of S3, 24.11 ± 18.07 percent of S4, 35.22 ± 17.61 percent of SWS, 10.67 ± 10.93 percent of REM.

Membership Recognition Task. Subjects from both groups learned stimuli group membership similarly. This is reflected in comparable number of trials to reach criterion (mean ± SEM: Nap = 1.93 ± 0.21; No-nap = 1.93 ± 0.18), hit rate (Nap = 85.33 ± 4.01 percent; No-nap = 89.33 ± 3.00 percent), false alarm rate (Nap = 17.33 ± 3.84 percent; No-nap = 28.00 ± 5.54 percent), and accuracy (hit rate – false alarm rate: Nap = 68.00 ± 5.71 percent; No-nap = 61.33 ± 5.76 percent). Independent t test found no significant group difference in these measures (all p values < 0.13).

Relational Memory Task. Data from this version of the task were analyzed with baseline performance achieved on the membership recognition task as a covariate to in order to control for the subjects' general ability to encode the stimuli as well as general tendency to have false alarms. Particularly, hit rate in the membership recognition task served as the covariate when hit rate in the relational memory task was the dependent variable. Respectively, false alarm rate in the membership recognition task served as the covariate when false alarm rate in the relational memory task was the dependent variable.

On both days of study, the two groups showed no difference in performance on the associative memory questions: match and non-related non-match (One-way ANCOVA, all p values > 0.13). Of the relational memory portion of the task, the spatially-related non-match questions did not reveal any group difference either (both p values > 0.18). On day 1, the nap subjects made significantly more false alarms on temporally-related non-match questions ($F_{1, 27}$ = 5.83, p = 0.02, η^2 = 0.18; mean ± SEM: Nap = 28.67 ± 4.77 percent; No-nap = 20.67 ± 3.71percent). After one week, this nap effect on temporally-related false alarms diminished (p = 0.83). Further investigation revealed this type of false alarms made by the no-nap subjects increased one week later (Paired samples t test, p = 0.048) while the nap subjects' responses remained relatively stable (p = 0.38, see *figure 3.3*). Numerically, the no-nap subjects had similar increase on spatially-related false alarms but it was not significant. On the contrary, the false alarm rate on the non-related non-match questions remained stable. Collectively, although the nap subjects had higher false alarm rate on the relational memory type questions (One-way ANCOVA , p = 0.10) immediately after the nap, the no-nap subjects ended with a comparable level of false alarm rate on these questions after one-week (Fixed effect ANCOVA, Group x Time of Testing interaction, $F_{1,27}$ = 4.28, p = 0.048, η^2 = 0.137).

Context Recall Task. Number of correct responses on recall of presentation order and location of individual pictorial stimuli were analyzed separately, using repeated-measures ANOVA with Group (Nap vs. No-nap) as the between-subject factor and Time (Day 1 vs. Day 2) as the within-subject factor . There was a clear decline in recall performance for both measures after a one-week period (Time main effects: both p values < 0.001). There was no Group main effect for each measure (both p values > 0.25). There was no Time x Group interaction for explicit recall of stimulus location (p = 0.22). On the contrary, there was a significant Time x Group interaction for recall of temporal order of stimulus ($F_{1, 28} = 4.04$, $p = 0.05$, $\eta^2 = 0.13$). Better retention of temporal information in the nap subjects appeared to drive this interaction. Subsequent independent t test showed the nap subjects retained a higher percentage of what the temporal information on day 2 (Dependent variable = number of correct responses on day 2 / number of correct responses on day 1; $t_{28} = 2.14$, $p = 0.04$; mean ± SEM: Nap = 0.77 ± 0.07; No-nap = 0.55 ± 0.07; see *figure 3.4*).

Sleep Stages and Memory. Unique contribution of individual sleep stages to memory processes were examined by the use of simultaneous linear-model regressions Criterion variables in these analyses were measures from the post-nap behavioral tasks and the predictors were duration of sleep stages: S1, S2, SWS (S3 + S4, as conventionally reported in the literature), REM.

For the relational memory task on day 1, seventy percent of variance of the nap subjects' accuracy (hit rate – total false alarm rate) is accounted for duration of individual sleep stages (p = 0.01). Noticeably, SWS has the most significant contribution although the partial correlation barely missed significance (pr = 0.56, p = 0.057). Other sleep stages do not correlate with accuracy (all p values > 0.17). Behavioral responses on the non-match questions were further decomposed into false alarm rate on the non-related (NR), false alarm rate on temporally related (TR), and false alarm rate on spatially related (SR) non-match questions. Variances of all three measures are considerably, though not all significantly, accounted for by a regression model composed of duration of sleep stages (NR: $R^2 = 0.63$, $p = 0.03$; TR: $R^2 = 0.47$, $p = 0.14$; SR: $R^2 = 0.54$, $p = 0.08$). Interestingly, duration of SWS inversely correlates with false alarm rate on all three types of non-match questions (NR: pr = -0.59, p = 0.046; TR: pr = -0.59, p = 0.043; SR: pr = -0.68, p = 0.016; see *figure 3.5*). These results reflect a negative relationship between SWS and subjects' overall tendency to make false alarms (total false alarm rate: pr = -0.77, p = 0.003; see *figure 3.5e*). S2 and REM did not correlate with any of the behavioral variables. S1, on the other hand, showed significant negative correlations with total false alarm rate (pr = -0.60, p = 0.041) and false alarm rate on SR non-match questions (pr = -0.58, p = 0.047; see *table 3.2* for a summary of partial correlations and significance levels). However, unlike SWS, S1 duration does not consistently correlate with all types of false alarm rates. Taken together with the fleeting and transitional nature of S1, these significant correlations warrant caution in interpretation.

For the relational memory task on day 2, the predictability of behavioral measures based on duration of sleep stages dropped, with no significant model fit found (R^2 ranging from 0.12 to 0.41). SWS no longer correlates with most of the behavioral measures, except for false alarm rate on the non-related non-match questions (pr = -0.59, p = 0.045). None of the other sleep stages correlates with the accuracy, or any of the false alarm rates.

Residuals in all were normally distributed (One-Sample Kolmogorov-Smirnov Test, all p values > 0.84). The assumption of homoscedasticity was also met as all correlation between duration of the individual sleep stages and the standardized residuals were non-significant (p > 0.99).

Discussion

The present study investigated the immediate and long-term effect of a daytime nap on integrating temporal and spatial relations of stimuli presented as belonging to separate groups. Relational memory here is reflected in false alarm rates on temporally-related and spatially-related non-match questions when subjects were making group membership judgments on stimuli that did not belong to the same triplet group but shared either the same presentation order or location. In brief, daytime napping had an immediate effect on relational memory, but only on the temporally-related non-match questions. Specifically, the nap subjects made significantly more false alarms on these questions after napping. The effect was no longer apparent after a one-week delay. Interestingly, after one week, while the nap effect on temporally-related false alarms diminished, the nap subjects had significantly better retention of the presentation order of individual pictures. SWS correlated positively with accuracy on membership recognition and negatively with general tendency to make false alarms.

The findings paint a more complex picture than the *a priori* hypotheses. There are several focal points that deserve particular attention.

Immediate effect of daytime napping

The immediate moderating effect of daytime napping on context-dependent formation of relational memory is one focal point of the present study. Here, context is operationalized as two constituents – temporal and spatial information associated with each stimulus. False alarm rates on both the temporally-related and spatially-related non-match questions are considered reflections of relational memory. However, an immediate nap effect is observed only in the temporally-related items.

The moderating effect of daytime napping on relational memory founded on temporal context is consistent with the existing literature. A plethora of existing research found the hippocampus to be central to integrating sequential order of events (Fortin et al., 2002; Kesner et al., 2002; Ergorul & Eichenbaum, 2006; Manns et al., 2007). For example, selective hippocampal lesion impaired memory of sequences ((Fortin et al., 2002; Kesner et al., 2002; Ergorul & Eichenbaum, 2006) while successful memory of stimuli order was dependent on gradual change in the pattern of hippocampal activity (Manns et al., 2007). At the same time, the hippocampus is known to be operative during sleep (Buszaki, 1998; Peignaux et al., 2003). More telling, reactivation of hippocampal cell population during sleep maintains the temporal relationship of their relative peak spiking time as during wakefulness (Wilson & McNaughton, 1994; Louie & Wilson, 2001; Lee & Wilson, 2002). Behaviorally, sleep preferentially facilitates recall of unrelated words in the original presentation order (Drosopoulos, 2007). In addition, a secondary finding in the present study corroborates with the immediate nap effect on temporally-based relational memory. Namely, the retention of presentation order after a one-week delay benefited from post-learning napping. Therefore, taken together, the findings suggest even a brief daytime nap can promote the integration of temporal information from discrete events into a relational memory network perhaps through hippocampal activities subserving such memory processes.

Careful reevaluation of existing literature and the current task offers explanations for the discrepancy between responses on temporally and spatially related non-match questions. The spatial context in the current task is the quadrant in which the stimulus appeared. It is different from the experience of spatial context when one navigates an environment. In fact, learning routes from a ground perspective activates the parahippocampus and the hippocampus whereas learning through a bird’s-eye-view survey activates the inferior temporal and posterior parietal cortex (Shelton & Gabrieli, 2002). Two behavioral studies reported a sleep or nap effect on spatial memory. In one, spatial learning was achieved through egocentric navigation of a 3D environment (Wamsley et al., 2010). In the other, spatial location was explicitly part of the associations learned (Talamini et al., 2008). In comparison, the spatial element in the current task might have engaged brain systems different from those subserving relational memory. Another possibility emerges considering reports from neuro-imaging studies, which found sleep-dependent changes in neural substrates, but not behavioral manifestation, of spatial memory (Peigneux et al., 2004; Rauchs et al., 2008). In the present study, napping may also lead to systematic changes in neural correlates of spatially-based relational memory without drastically changing the behavioral response supported by them. Therefore, the discrepancy between temporally and spatially related false alarms in the present study is not as striking as it first appears.

There is no immediate nap effect on hit rate or on false-alarm rate on the non-related non-match questions during membership recognition, seemingly out of harmony with some sleep studies on direct associative memory. However, closer examination of studies using recognition tasks presents an equivocal picture. Similar to the present study, others have also reported no positive sleep effect on recognition memory (Takashima et al., 2006; Sheth et al, 2009). Studies reporting small to moderate sleep effect on recognition memory either used far more stimuli (Mongrass et al, 2008 used 160 stimuli compared to 24 in the present study) or highly arousing stimuli (Wagner et al., 2007 used faces with emotional expressions compared to abstract pictures in the present study). The use of overnight sleep protocol and the lack of performance criterion at learning further differentiate these studies from the present one. Collectively, the observations suggest recognition tasks to be relatively less sensitive measurements of sleep-dependent changes in direct associative memory. These results do not diminish the significance of the present study because the element of direct associative memory here simply provides the foundation of, and is, secondary to the examination of relational memory. Moreover, the results imply that the real value of post-learning sleep may not be a quantitative change (i.e. strengthening of encoded materials) but a qualitative one (i.e. integration of encoded materials into a relational memory network).

Long-term effect of daytime napping

After one week, the group difference on temporally-based relational memory diminished. There are three possible explanations for this. Napping might have transiently influenced performance by altering arousal. In this case, however, one would expect to see changes in other measures such as hit rate as well as accuracy. Absence of such changes renders this possibility very unlikely. Alternatively, sleep obtained during the one-week delay might have compensated the no-nap subjects. Between the first and second testings, all subjects had an adequate amount of nocturnal sleep, evident from their sleep log entries (subjects on average reported having about eight hours of sleep the night prior to study day 2). The experimental post-learning nap was brief by comparison and could be the initial stage of relational memory processing. This interpretation implies that the delay between learning and sleep onset may not be critical to the merit of sleep-dependent memory processes. Corroborating with this notion, a few studies showed that sleep facilitates memory even when it occurs after learning with a substantial delay

(Fenn et al., 2003; Backhaus et al., 2007). Results in the present study are also in line with this explanation. Subjects without the post-learning nap had an increase in both temporally-related and spatially-related false alarms but not in non-related false alarms. Yet another possibility is that napping might have resulted in covert changes in representations of memory. Indeed, fMRI studies typically showed post-learning sleep resulting in a long-lasting shift in neural representations of memory, from the hippocampus at initial learning to other regions in the brain (Orban et al., 2006; Takashima et al., 2006; Gais et al., 2007). Divergent from the physiological results, none of these studies found visible behavioral difference after a long delay. Out of the three possibilities, results in the present study are better explained by the latter two or a combination of the two.

Notably, post-learning napping resulted in superior retention when subjects had to explicitly recall the original presentation order of individual pictures after one week. Together with the immediate nap effect on temporally-based relational memory, the findings suggest that sleep particularly benefits processing of temporal information. More important, the same temporal information appears to engage different memory processes during sleep. The result is not only stronger memory traces of stimulus presentation order but also a more elaborate memory network in which such temporal memories serve as nodes.

Sleep stages and memory processing

Consistent with the literature (Buszaki, 1998; Hasselmo, 1999; Sejnowski & Destexhe, 2000; Tononi & Cirelli, 2002, 2006), SWS appears to contribute the most to memory processes. It correlated positively with accuracy on membership recognition, supporting a role in direct associative memory processing. The negative correlations between SWS and temporally-related and spatially-related false alarms should not be interpreted superficially as slow-wave sleep being detrimental to relational memory. In fact, SWS negatively correlated with all types of false alarms, pointing to an influence on the overall tendency to make false alarms instead of an influence on relational memory per se. It would be premature to conclude that SWS has a link with the type of relational memory measured here or the nature of the link. As discussed earlier, the primary task in the present study may engage different sleep-dependent memory processes. In Study I, in which a positive correlation was found between SWS and the relational memory measure, the relational memory task and the associative memory task did not conflict with each other. In fact, they might have even complemented each other. On the contrary, the direct associative memory and the relational memory elements in the present study lead to conflicting responses (i.e. making accurate group membership recognition vs. making temporally-related and spatially-related false alarms). In addition, the subjects in the present study were explicitly instructed to memorize group membership but not presentation order or location of the stimuli._ Therefore, SWS might indeed contribute to relational memory in the present study but processes supporting group membership might have overpowered processes supporting temporally and spatially based relational memory.

Future research

The complex results present intriguing new directions for future research. Using a more salient spatial context, perhaps through an egocentric navigation experience at encoding, allows clarification of the involvement of sleep in spatial memory and spatially-based relational memory. Another interesting follow-up study would be to shift the task demand to remembering the presentation order and/or location of the stimuli, making group membership the context upon which relational memory can be built. Not only will this simple change in protocol be a necessary replication, but it will also help clarify the relationships SWS has with direct associative memory and relational memory, respectively. If SWS indeed has a dual role in contributing to two different memory processes simultaneously, there may be a reversal in the patterns of correlations between SWS and memory measures compared to the current findings. Finally, it is also important to collect physiological measures long after napping occurs to further elucidate the long-term effect of daytime napping on relational memory.

Overall Conclusion

The current series of studies demonstrate the facilitating effect of daytime napping on relational memory. The effect is generalized to a variety of tasks; all start with overt encoding of direct associative memory and each allows probing of a different form of relational memory. Measures of relational memory in these studies require processing beyond rote memorization of the explicitly encoded associations, implying an active rather than passive role of sleep in memory processes. At the same time, these studies underscore the complexity of this research topic. While behavioral measures from all three studies fall under the umbrella of relational memory, the fashion of establishing the indirect relational links varies due to formats of stimuli and premises of tasks. The relational memories from these studies may depend on different neural pathways and/or mechanisms. Therefore, the sleeping state may be important to multiple memory processes.

Among the sleep stages, slow-wave sleep (SWS) emerges as the most influential to memory performance with significant correlations in two of the three studies. There are several possible ways SWS may contribute to memory processes. First, physiological parameters unique to SWS may be mechanisms supporting memory (Buszaki, 1998). Second, neurochemical fluctuations during SWS may particularly favor system-level communication, aiding formation of elaborate and interconnected memory networks (Hasselmo, 1999). Third, homeostatic regulatory processes may occur in SWS, resulting in pruning of trivial information and relative strengthening of more pertinent experiences (Tononi & Cirelli, 2002). These possibilities are not mutually exclusive and thus each may be partially responsible for the current findings. Granted that SWS stands out as the foremost contributor to memory performance, it alone cannot fully explain the effect of sleep on relational memory tasks. This point is highlighted by the complex patterns of correlation results across the three studies. Future research should expand the investigation beyond sleep-stage-dependent parameters. Meanwhile, better understanding of different forms of relational memory is necessary in order to better discern the nature of the involvement of sleep in each memory process.

It should not be overlooked that results in the current studies were obtained after only a relatively brief period of daytime sleep, consisting of about fifty to seventy minutes of total sleep time. It is quite possible that relational memories are even more impacted by longer durations of nocturnal sleep. In any case, the fact that a short bout of sleep has the ability to produce significant differences in memory performance is impressive. Our culture encourages the sacrifice of sleep time for productivity, and therefore considers napping counterproductive. On the contrary, as results in the current studies indicate, daytime napping facilitates reorganization of memory to allow flexible manifestation upon novel task demands at a later time.

Significance of the Present Research

On a final note, the present work has obvious practical applications to the facilitation of learning experiences in occupational situations wherein full night sleep availability is limited, such as in the military. In addition, examining sleep parameters that may contribute to relational memory may lend further understanding to the neural basis of memory processes in populations with memory dysfunctions, such as amnesic (Kan et al., 2007; Konkel et al., 2008) and schizophrenic patients (Titone et al., 2004), both of which exhibit severe deficits in relational memory. Schizophrenics, in particular, also frequently have difficulties initiating and maintaining sleep (Monti & Monti, 2005). The present research may lay ground for utilization of sleep parameters as nonintrusive early markers for exhibition of memory dysfunctions in these populations. Structuring a sleep or nap schedule with optimal efficiency for memory processing may be an effective behavioral intervention that can either facilitate memory retention in normal populations or alleviate memory dysfunctions with few side effects.

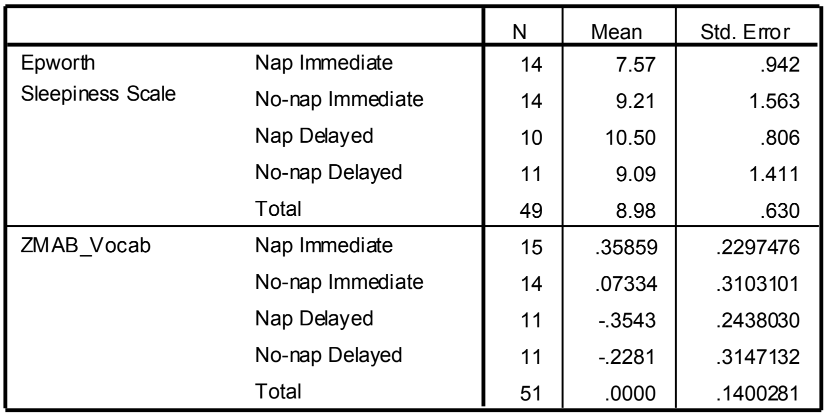
Table 1.1 Summary of group means and SEMs of sleepiness and intelligence measures.

	group (nap/no-nap)	N	Mean	Std. Error Mean
Epworth Sleepiness Scale	Nap	18	9.11	.896
	No-nap	17	8.06	.851
Raw score of MAB Arithmetic	Nap	18	11.06	.623
	No-nap	17	10.71	.674
Raw score of MAB Picture Completion	Nap	18	14.44	1.294
	No-nap	17	12.76	1.006
Raw score of MAB Picture Arrangement	Nap	18	9.44	.668
	No-nap	17	9.82	.523

Table 1.2 Summary of group means and SEMs of response time on the tasks.

	group (nap/no-nap)	N	Mean	Std. Error Mean
response time ab1	Nap	18	0:05:50.68	0:00:19.7
	No-nap	17	0:05:44.13	0:00:17.8
response time bc1	Nap	18	0:06:21.20	0:00:28.9
	No-nap	17	0:05:33.56	0:00:14.6
response time ab2	Nap	18	0:05:28.95	0:00:14.1
	No-nap	17	0:05:23.21	0:00:20.9
response time bc2	Nap	18	0:05:24.97	0:00:18.3
	No-nap	17	0:05:08.32	0:00:23.6
response time ac	Nap	18	0:10:15.68	0:00:47.2
	No-nap	17	0:09:31.23	0:00:37.8

Table 2.1 Summary of group means and SEMs of sleepiness and intelligence measures.



		N	Mean	Std. Error
Epworth Sleepiness Scale	Nap Immediate	14	7.57	.942
	No-nap Immediate	14	9.21	1.563
	Nap Delayed	10	10.50	.806
	No-nap Delayed	11	9.09	1.411
	Total	49	8.98	.630
ZMAB_Vocab	Nap Immediate	15	.35859	.2297476
	No-nap Immediate	14	.07334	.3103101
	Nap Delayed	11	-.3543	.2438030
	No-nap Delayed	11	-.2281	.3147132
	Total	51	.0000	.1400281

Table 2.2 Summary of group means and SEMs of response time on the tasks.

		N	Mean	Std. Error
Response Time: Cued Recall Task	Nap Immediate	15	0:03:53	0:00:19
	No-nap Immediate	14	0:04:36	0:00:15
	Nap Delayed	11	0:04:49	0:00:41
	No-nap Delayed	11	0:04:58	0:00:39
	Total	51	0:04:31	0:00:14
Response Time: Multiple Choice Task	Nap Immediate	15	0:06:12	0:00:20
	No-nap Immediate	14	0:06:05	0:00:27
	Nap Delayed	11	0:07:26	0:00:38
	No-nap Delayed	11	0:05:43	0:00:38
	Total	51	0:06:20	0:00:15
Response Time: Radical Task	Nap Immediate	15	0:02:35	0:00:12
	No-nap Immediate	14	0:02:26	0:00:11
	Nap Delayed	11	0:03:27	0:00:29
	No-nap Delayed	11	0:02:53	0:00:25
	Total	51	0:02:47	0:00:09

Table 2.3 Summary of sleep parameters across the two nap groups (immediate vs. delayed). Asterisk represents significant group difference (* $p < 0.05$)

	Immediate or Delayed	N	Mean	Std. Error Mean
Sleep onset	Nap Immediate	15	8.6333	1.18369
	Nap Delayed	11	6.8182	2.21079
Sleep efficiency (%)	Nap Immediate	15	79.93	2.89235
	Nap Delayed	11	80.82	3.50449
Totoal sleep time *	Nap Immediate	15	63.17	2.92756
	Nap Delayed	11	77.45	3.80637
Stage 1	Nap Immediate	15	5.9333	1.30646
	Nap Delayed	11	6.2727	.91791
Stage 2	Nap Immediate	15	31.33	2.59334
	Nap Delayed	11	36.09	4.32741
Stage 3	Nap Immediate	15	6.6000	1.39233
	Nap Delayed	11	7.0909	1.11563
Stage 4	Nap Immediate	15	13.17	3.74791
	Nap Delayed	11	15.09	3.94670
Slow wave sleep	Nap Immediate	15	19.77	4.10745
	Nap Delayed	11	22.18	4.02903
REM sleep	Nap Immediate	15	5.6667	1.93383
	Nap Delayed	11	12.86	3.84654
Density_S1	Nap Immediate	15	.1019	.02375
	Nap Delayed	11	.0848	.01381
Density_S2	Nap Immediate	15	.5017	.04121
	Nap Delayed	11	.4697	.05850
Density_S3	Nap Immediate	15	.1019	.02084
	Nap Delayed	11	.0918	.01420
Density_S4	Nap Immediate	15	.1996	.05345
	Nap Delayed	11	.1984	.06043
Density_SWS	Nap Immediate	15	.3015	.05710
	Nap Delayed	11	.2902	.05894
Density_REM	Nap Immediate	15	.0881	.03069
	Nap Delayed	11	.1547	.04651

Table 3.1 Summary of group means and SEMs of sleepiness, motivation, and intelligence measures.

	Nap or No-Nap	N	Mean	Std. Error Mean
Epworth Sleepiness Scale	Nap	15	8.67	.924
	No-nap	15	10.13	.935
Motivation Scale	Nap	15	3.940	.1182
	No-nap	15	3.580	.1870
MABII - Vocabulary	Nap	15	17.33	1.132
	No-nap	15	14.73	1.119
MABII - Picture Completion	Nap	15	18.80	.962
	No-nap	15	16.67	1.333

Table 3.2 Partial correlations between duration of individual sleep stages and memory measures at testing on study day 1, with corresponding significance levels. Accuracy = hit rate – total false alarm rate; NR false alarm rate = false alarm rate on the non-related non-match questions; TR false alarm rate = false alarm rate on the temporally-related non-match questions temporally-related; SR false alarm rate = false alarm rate on the spatially-related non-match questions.

Sleep Stages	**Accuracy**		**NR False Alarm Rate**		**TR False Alarm Rate**		**SR False Alarm Rate**		**Total False Alarm rate**	
	Sig.	***Pr***	***Sig.***	***Pr***	***Sig.***	***Pr***	***Sig.***	***Pr***	***Sig.***	***Pr***
S1	.579	-.179	.659	-.142	.124	-.057	.047	-.583	.041	-.595
S2	.216	-.385	.429	.252	.955	.351	.556	-.189	.978	.009
SWS	.057	.563	.046	-.585	.043	-.541	.016	-.675	.003	-.770
REM	.169	.425	.878	-.050	.366	-.031	.348	-.297	.279	-.340

Figure 1.1

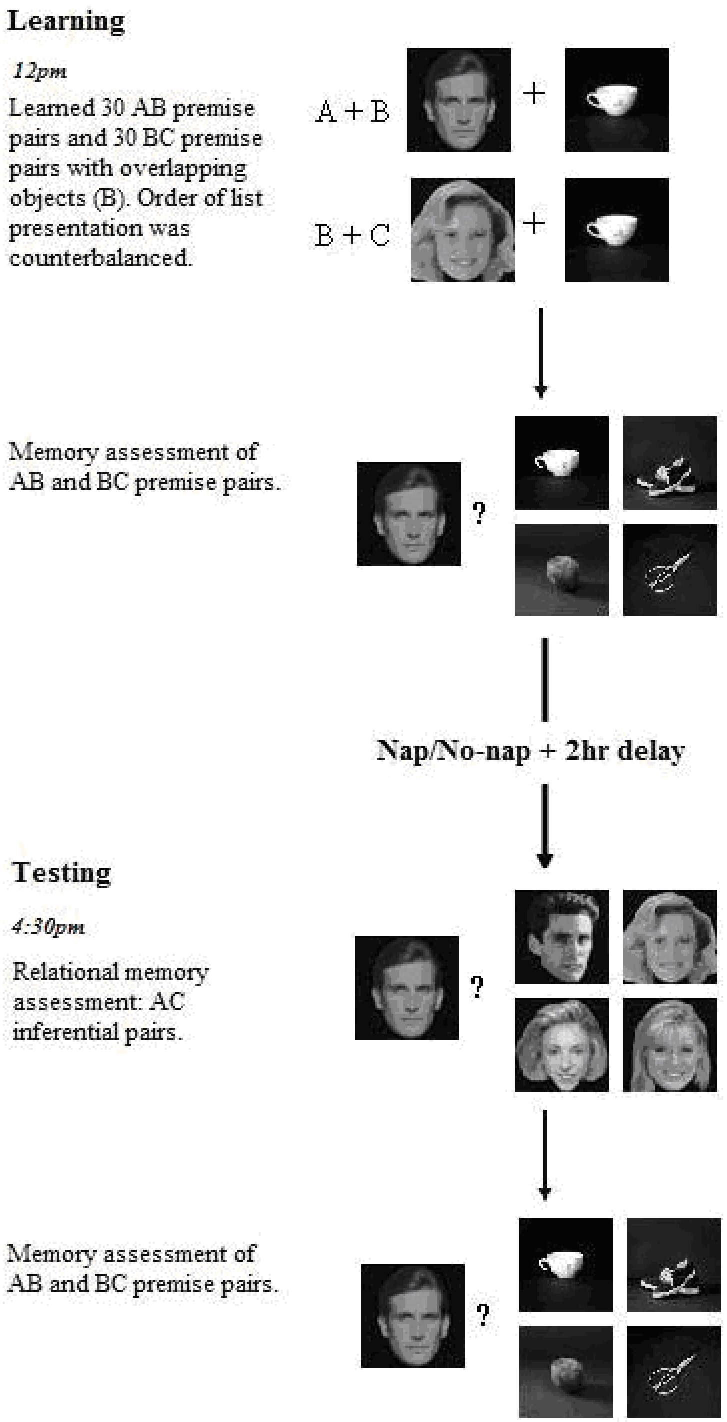


Figure 1.1. Summary of experimental procedure. Subjects were first exposed to two lists of face-object photograph pairs (AB and BC pairs) separately and were given a surprise face-face force-choice probe (AC pairs) during the testing session.

Figure 1.2

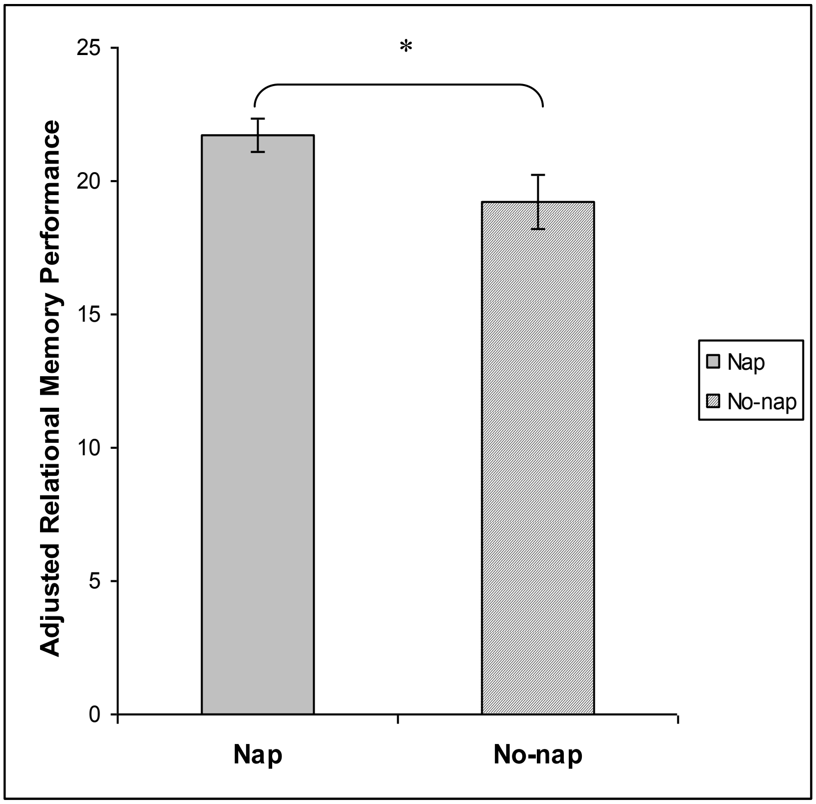


Figure 1.2. Adjusted relational memory performance across groups. Performance was adjusted by subtracting the difference between the raw score on the AC (face-face) task and the average score on the direct associative memory (face-object) tasks at learning from the maximum score (30) on the AC task. Asterisk represents significant performance difference (* $p < 0.05$).

Figure 1.3a

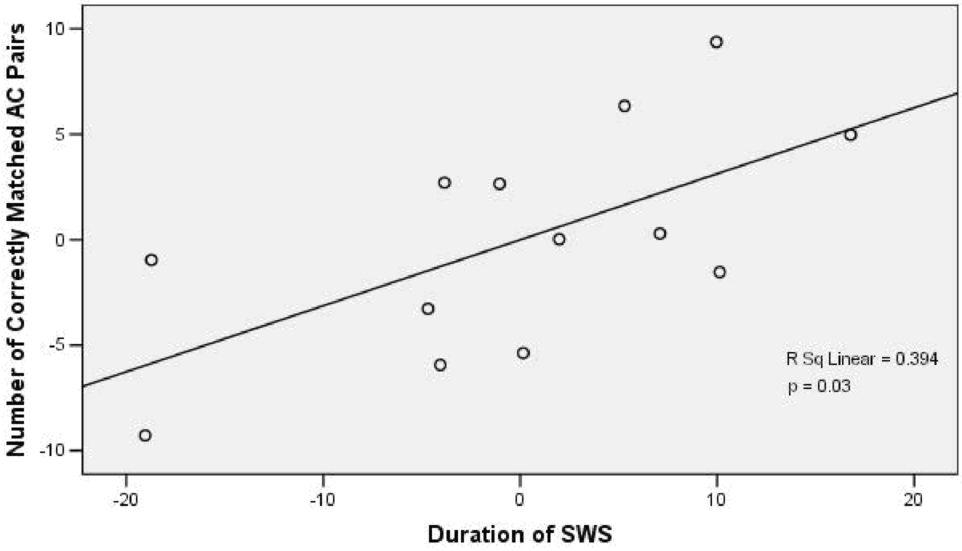


Figure 1.3b

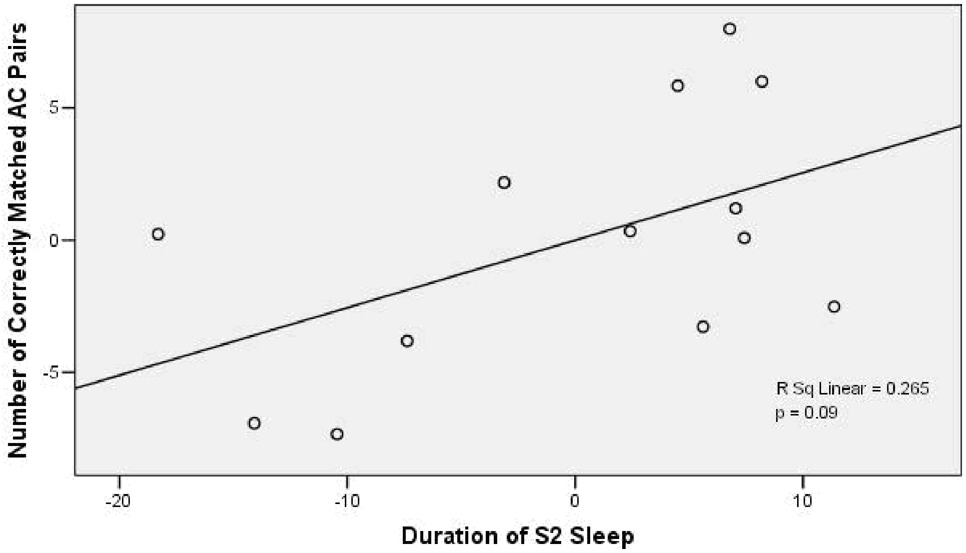


Figure 1.3. Partial regression plots indicating the relationship between relational memory and individual sleep stages, derived from a regression model with relational memory (i.e. the number of correctly matched AC pairs) as the criterion variable, and duration of S2 and SWS as predictor variables. *1.3a) Relationship between relational memory and SWS.* Y-axis represents residuals from regressing relational memory against S2 but not SWS. X-axis represents residuals from regressing duration of SWS against duration of S2. *1.3b) Relationship between relational memory and S2.* Y-axis represents residuals from regressing relational memory against SWS but not S2. X-axis represents residuals from regressing duration of S2 against duration of SWS.

Figure 2.1a

Figure 2.1b

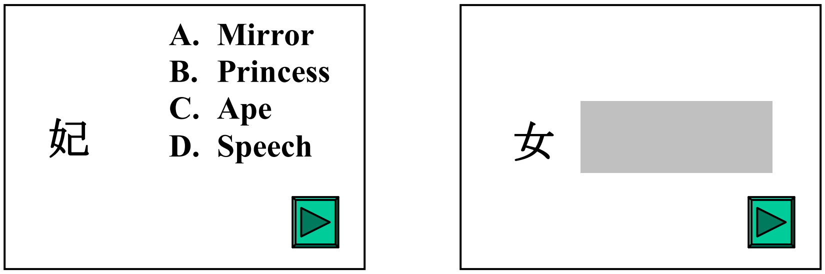


Figure 2.1. Sample stimuli and items from the tasks. *2.1a)* Individual characters, sharing the same radical, presented in the learning phase. *2.1b)* Left: A new character with a previously seen radical in the multiple-choice task. Right: An isolated radical in the radical task.

Figure 2.2

Group	11:30am	12:00pm	1:30pm	2:00pm	3:30pm	4pm
Immediate Nap (n = 15)	Learn	Nap condition		Test		
Immediate No-nap (n = 14)	Learn	No-nap condition		Test		
Delayed Nap (n = 11)	Learn	Video		Nap condition		Test
Delayed No-nap (n = 11)	Learn	Video		No-nap condition		Test

Figure 2.2. Summary of experimental design and protocol.

Figure 2.3

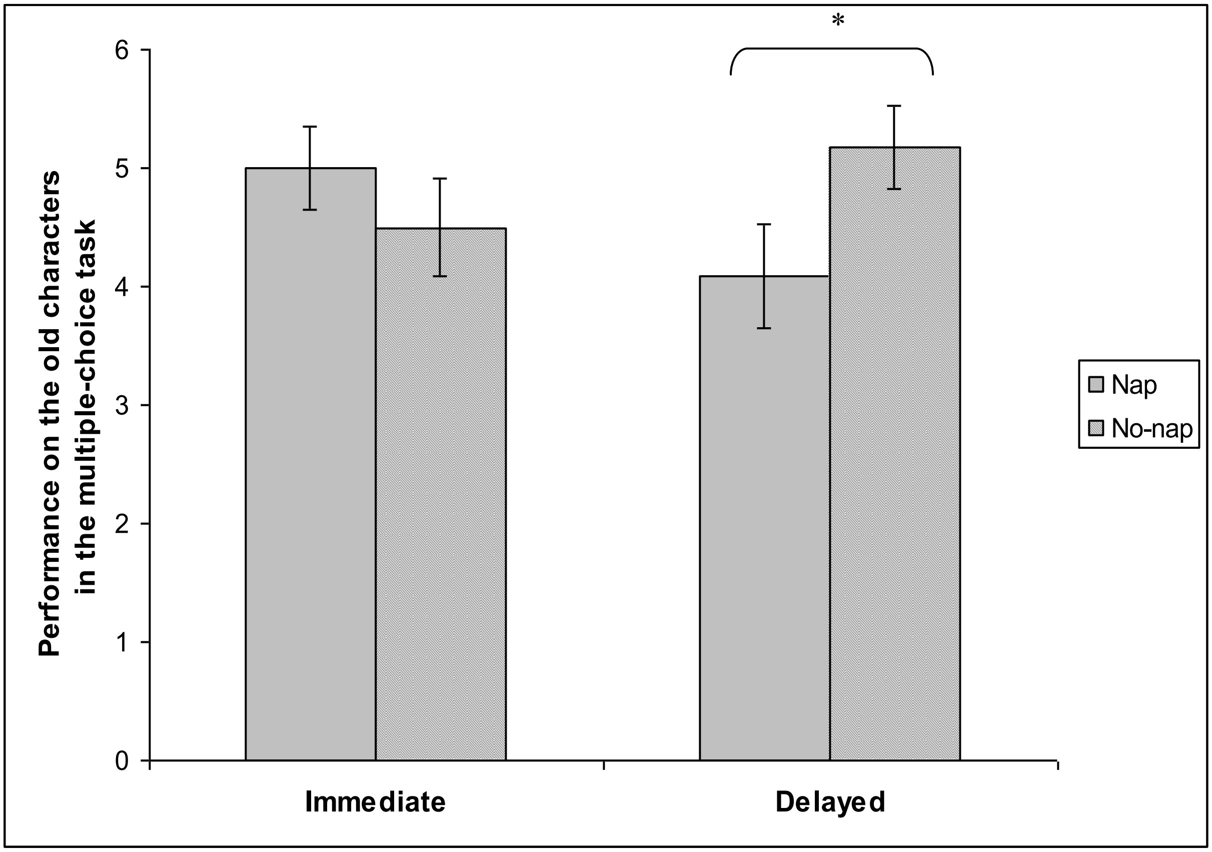


Figure 2.3. Number of correct answers on matching old characters to their English meanings in the multiple choice task. Asterisk represents significant group difference (* $p < 0.05$).

Figure 2.4

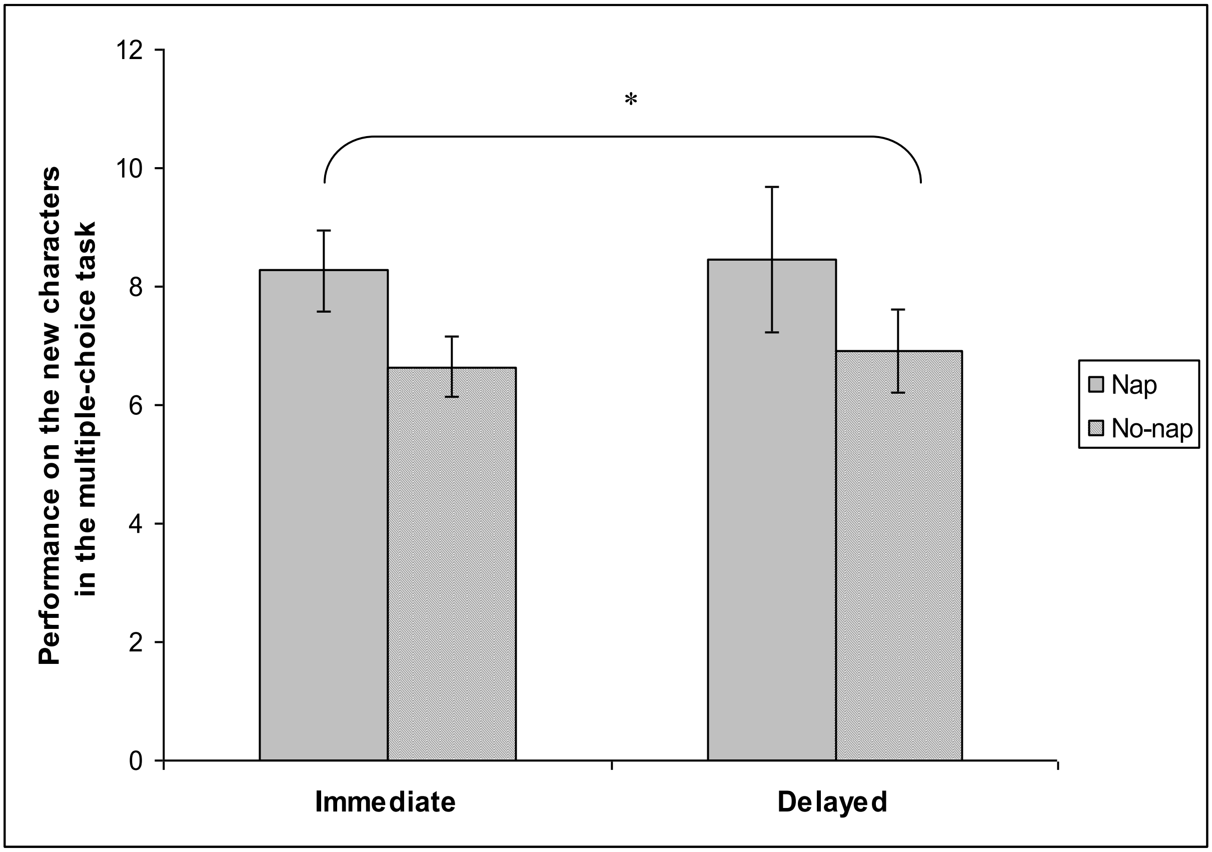


Figure 2.4. Number of correct answers on matching new characters to their English meanings in the multiple choice task. Asterisk represents significant main effect for the nap condition (nap vs. no-nap, * $p < 0.05$).

Figure 2.5

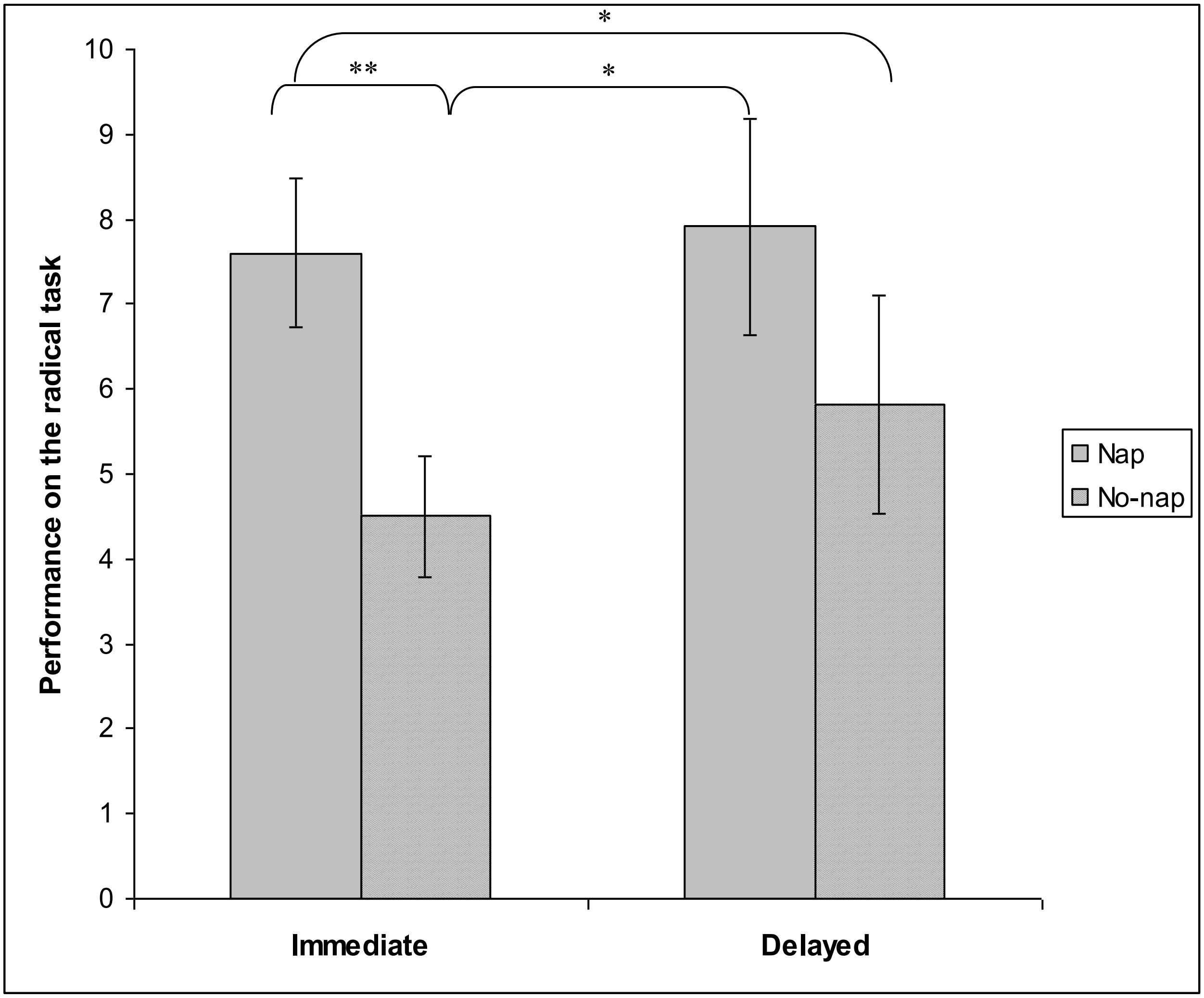


Figure 2.5. Performance on the radical task, in which subjects explicitly stated the general concepts represented by isolated radicals. Asterisks represent significant performance difference across groups (* $p < 0.05$, ** $p < 0.01$).

Figure 3.1

Figure 3.1a. Summarized procedure for the behavioral tasks and the experimental design.

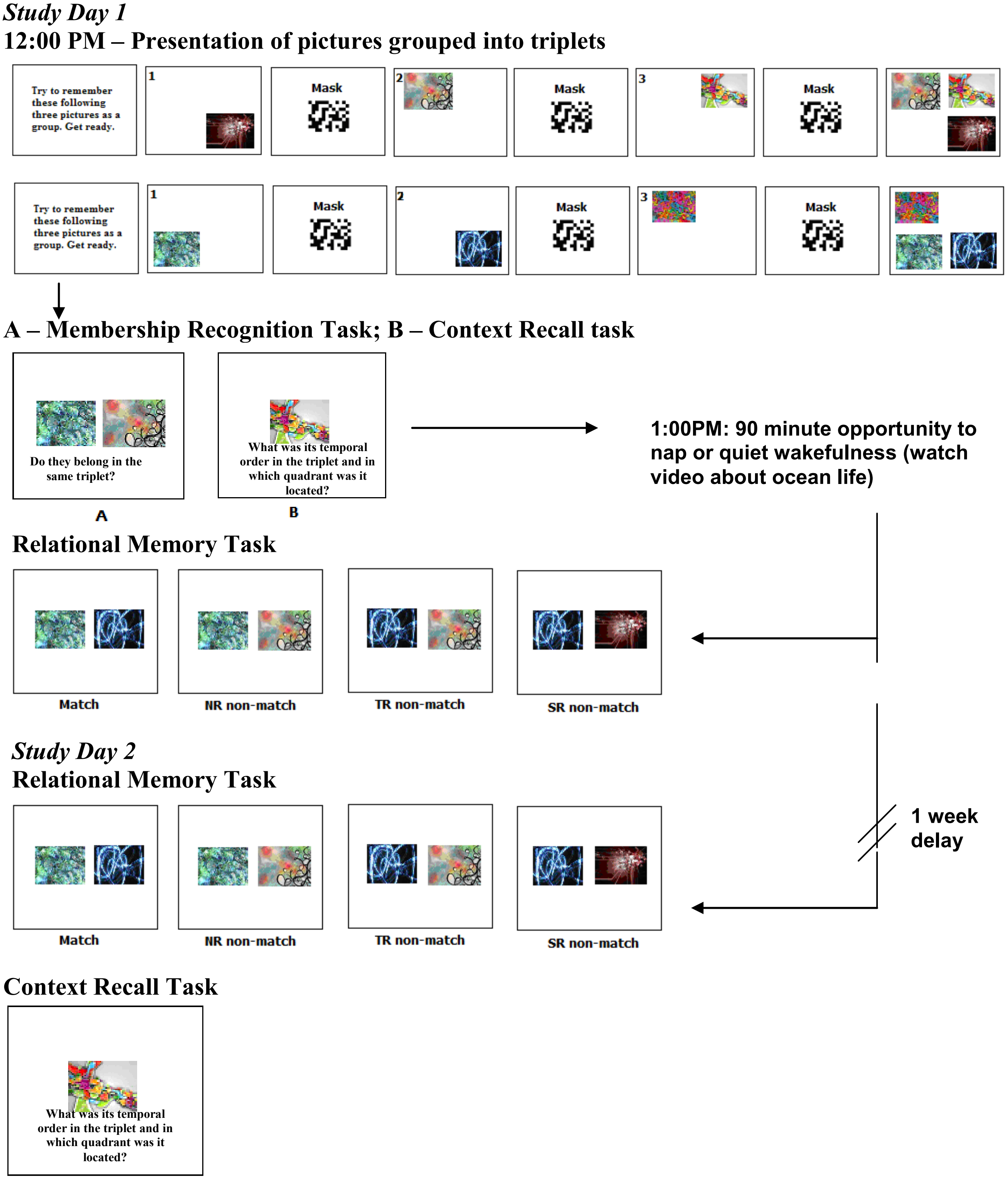


Figure 3.1b. The relational memory task consisted of four types of questions. The match and non-related non-match questions reflected direct associative memory. The temporally and spatially related non-match questions reflected relational memory.

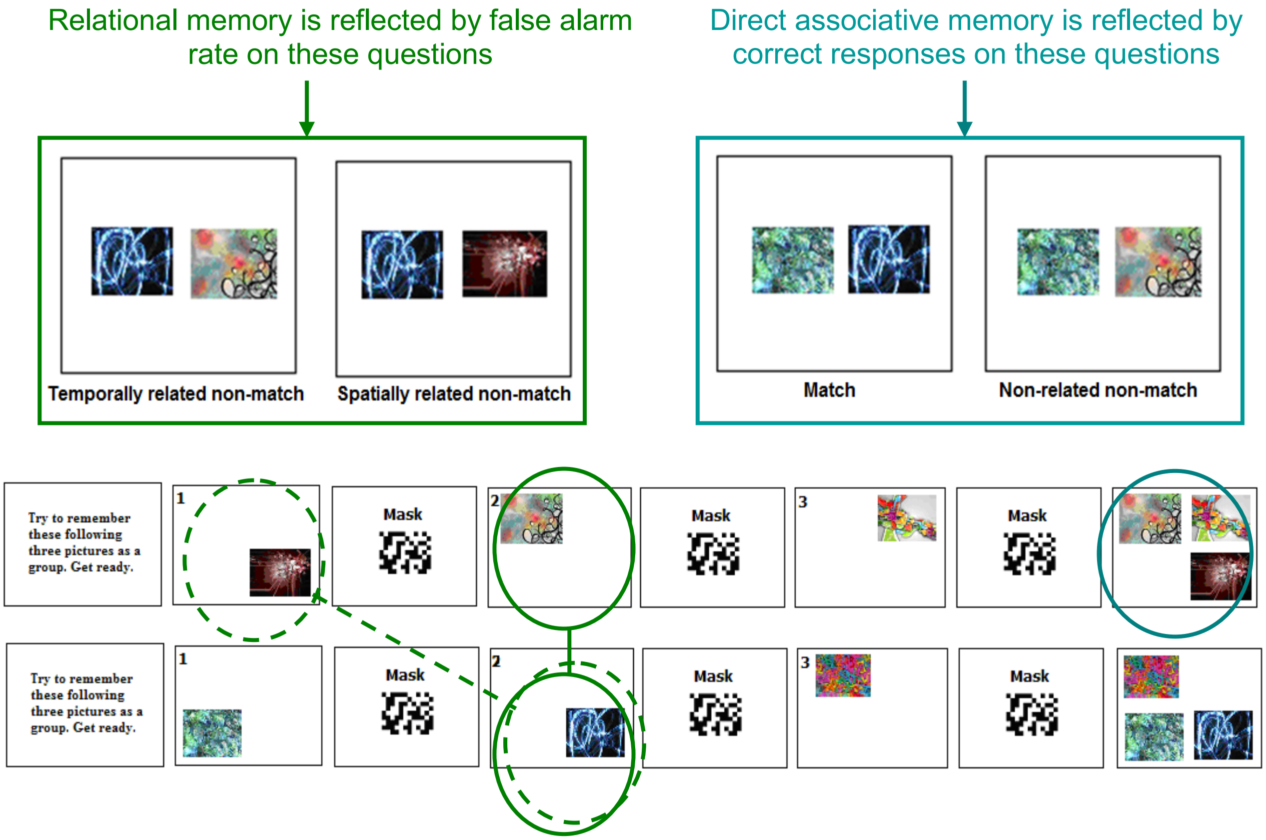


Figure 3.2. Subjects wore wrist bands when they went to bed each night during the one-week delay. They were told that it was a device that would document their sleep activity. The purpose of this was to increase compliance to maintain a regular sleep schedule and have adequate amount of sleep during the one-week delay.

Figure 3.3.

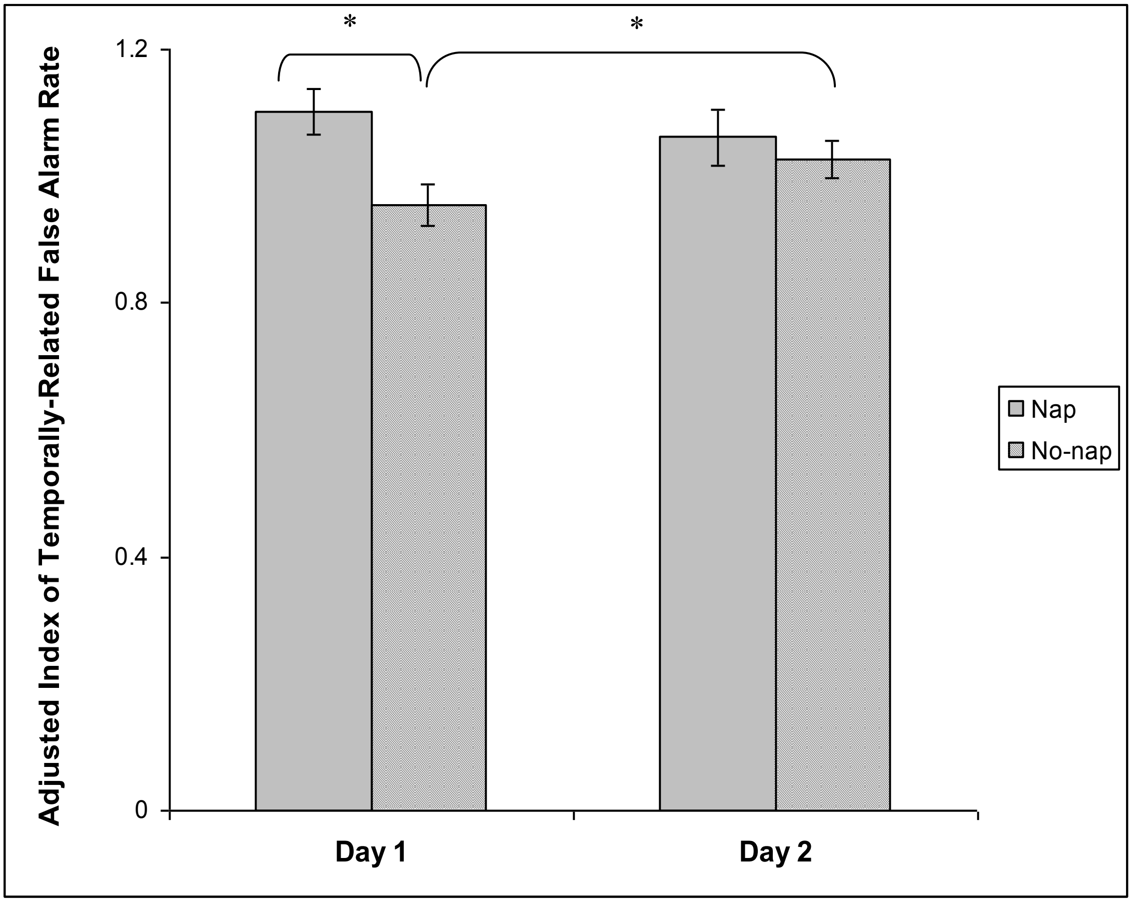


Figure 3.3. False alarm rate on the temporally-related non-match questions, adjusted to the general tendency of making false alarms ([TR false alarm rate + 1] / [false alarm rate in the membership recognition task given immediately after learning + 1]). . Asterisks represent significant difference across groups (* $p < 0.05$).

Figure 3.4.

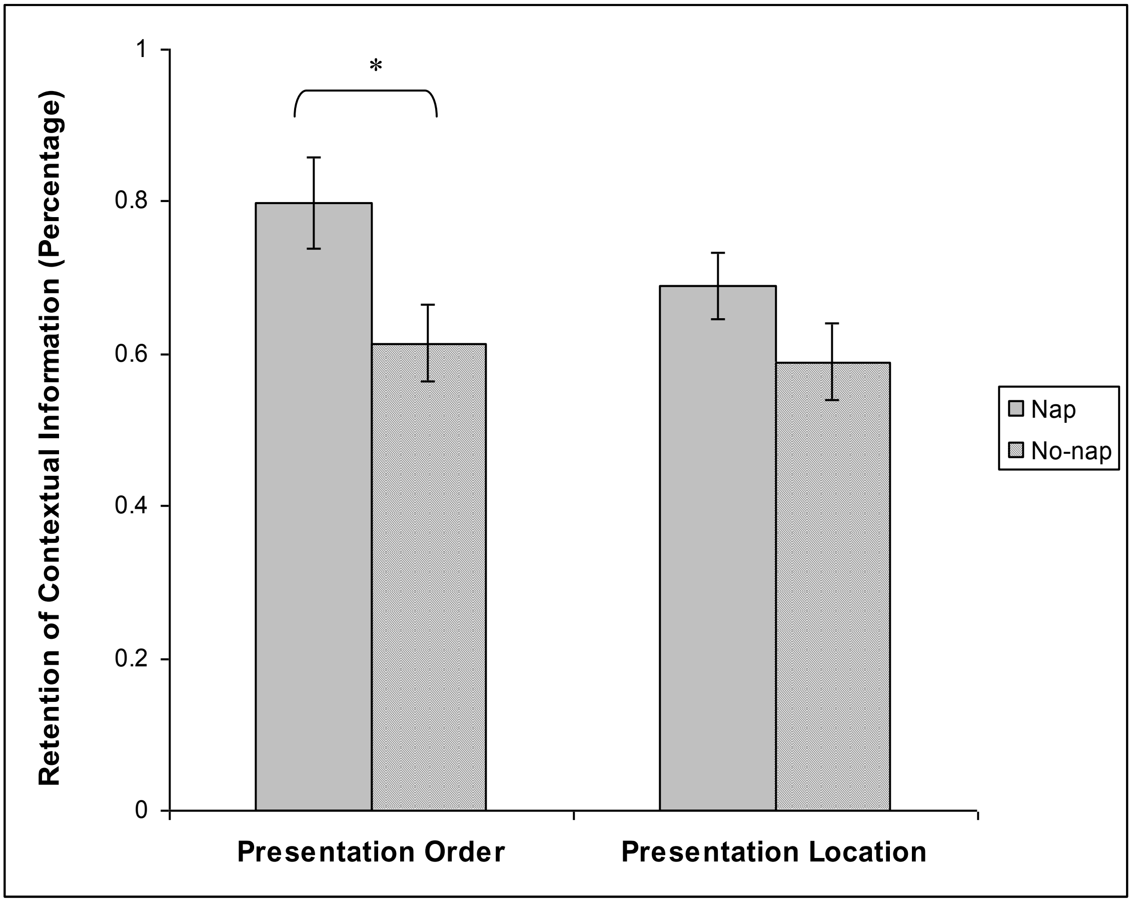


Figure 3.4. Retention of contextual information (i.e. original presentation order and location of a given picture). The Y axis represents the percentage of information retained (i.e. number of correct responses on day 2 / number of correct responses on day 1). Asterisk represents significant difference across groups (* $p < 0.05$).

Figure 3.5. Partial regression plots indicating the relationships between SWS and a) hit rate, b) false alarm rate on NR questions, c) false alarm rate on TR questions, d) false alarm rate on SR questions, and e) total false alarm rate in the relational memory task given on study day 1. Axes represent respective residuals from regression models.

Figure 3.5a

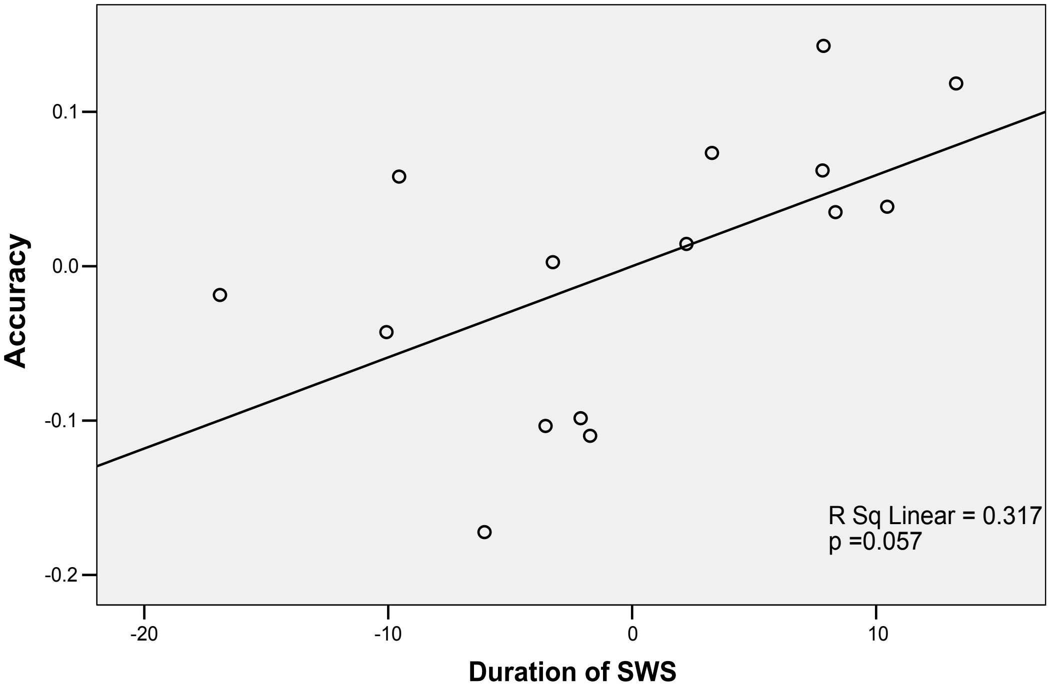


Figure 3.5b

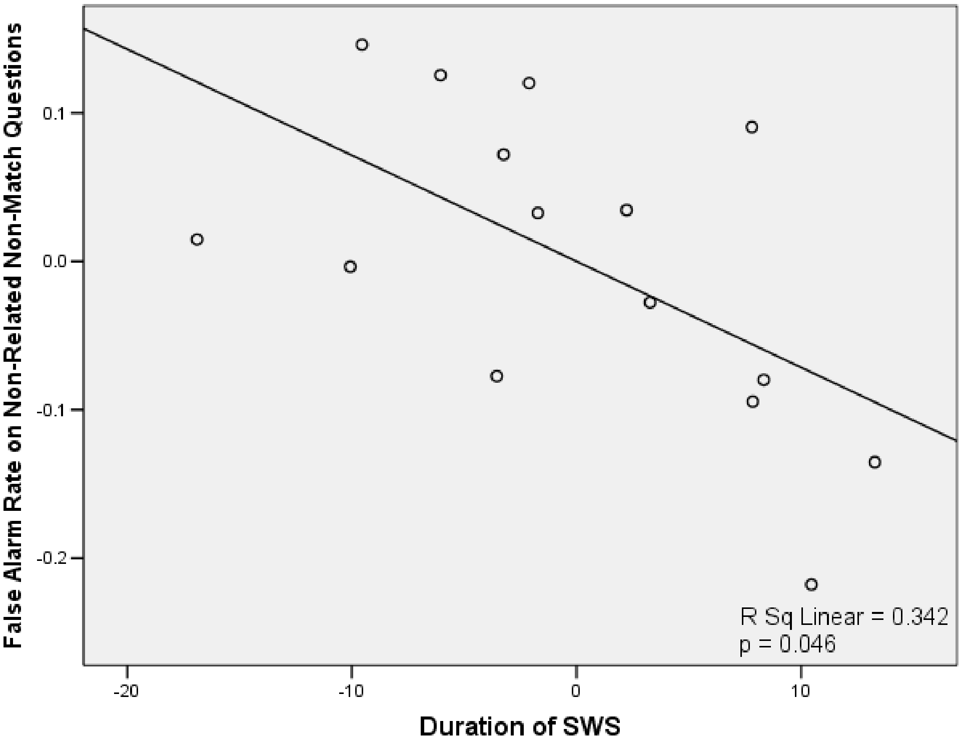


Figure 3.5c

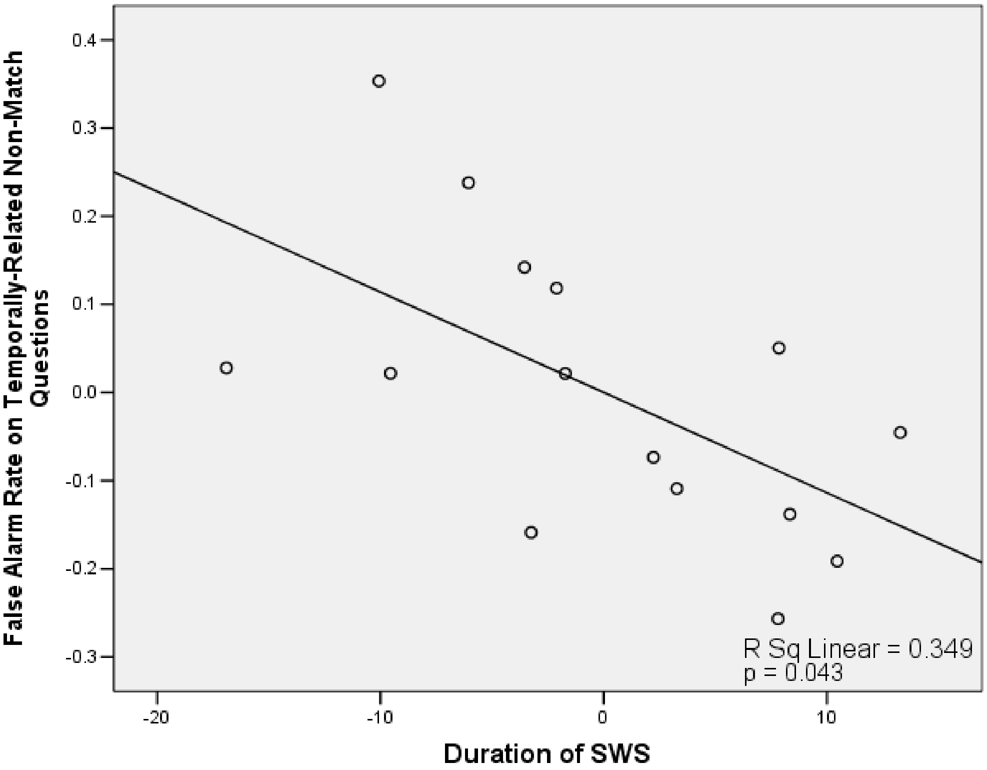


Figure 3.5d

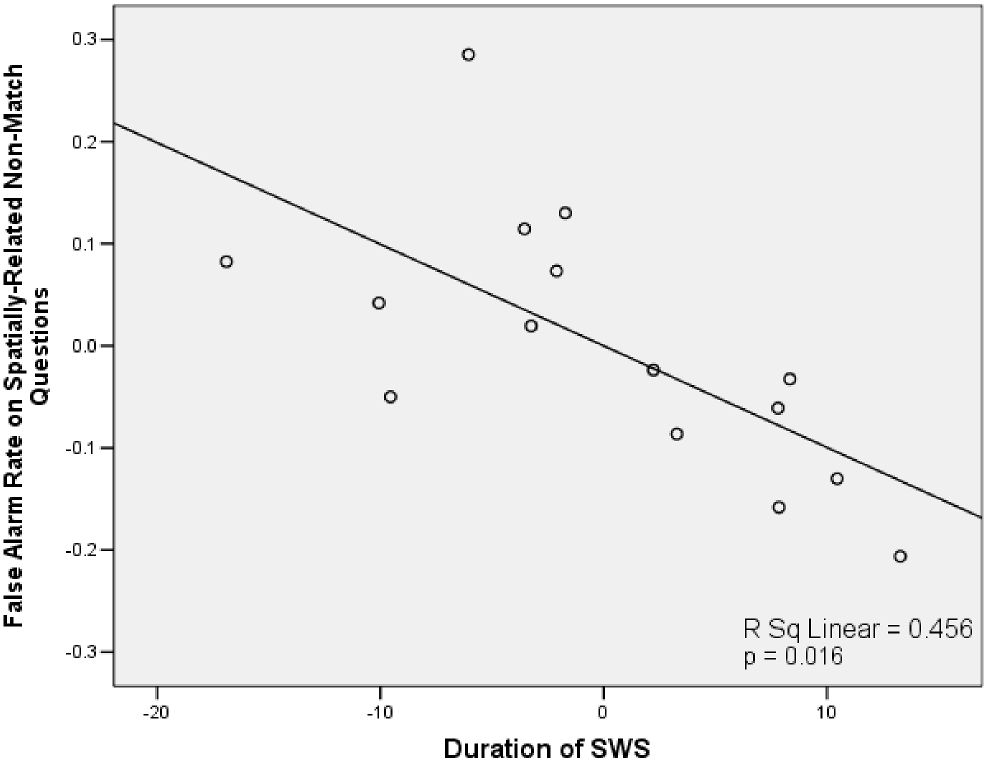
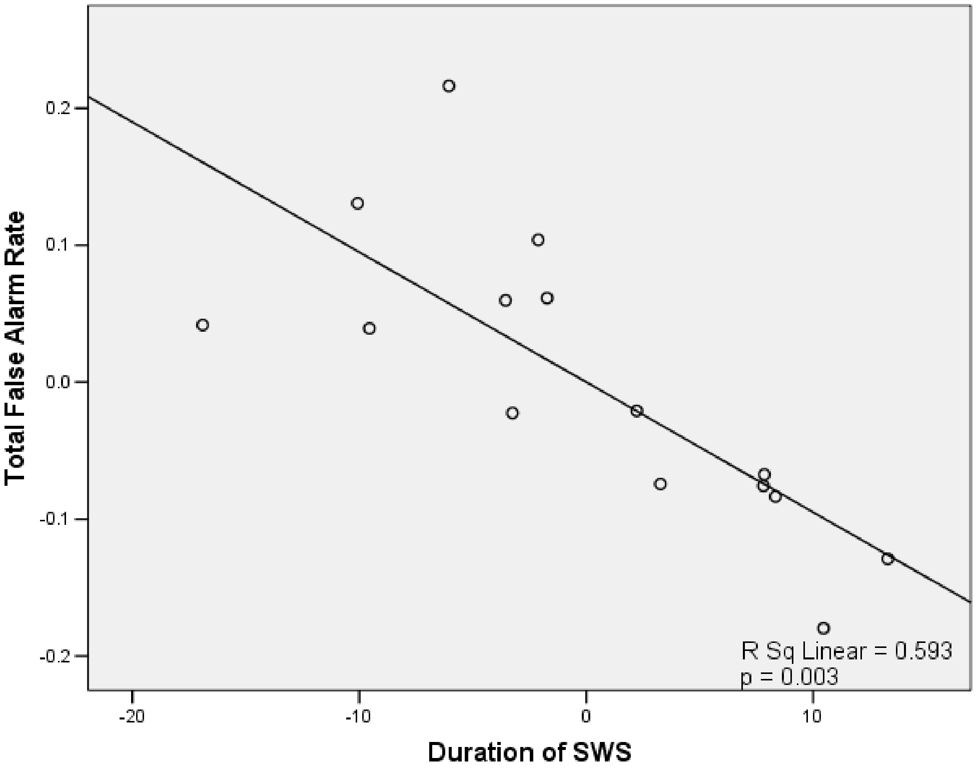


Figure3.5e



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