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Evidence for sleep-dependent memory consolidation in human and mice

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Evidence for Sleep-Dependent Memory Consolidation in Humans and Mice

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Psychology

by

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2010
DEDICATION

I would like to dedicate my dissertation to my mom and dad, Mona and Paul Cai.
I would not have been able to pursue my dreams without their unconditional support and profound sacrifices.
EPIGRAPH

“Eh, it’s not an exact science.”

*Stephan G. Anagnostaras*

“Your research should be sexy AND sound.”

*Michael R. Gorman*

“You should always be nice to people.
You never know when you’ll meet them next.”

*Sara C. Mednick*
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Chapter 1, in full, is a reprint of the material as it appears in Sleep-deprivation and Pavlovian fear conditioning. Learning & Memory. 16(10), 595-599. Cai, D.J., Shuman, T., Harrison, E.M., Sage, J.R., & Anagnostaras, S.G. (2009). The dissertation author was the primary investigator and author of this paper.


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Field of Study

Major Field: Psychology
Sub-fields: Neuroscience & Behavior
ABSTRACT OF DISSERTATION

Evidence for Sleep-Dependent Memory Consolidation in Humans and Mice

by

Denise Jade Cai

Doctor of Philosophy in Psychology

University of California, San Diego, 2010

Professor Stephan G. Anagnostaras, Chair

Professor Michael R. Gorman, Co-Chair

Sleep has been implicated as playing an important role in memory consolidation. Considerable indirect evidence suggests a role for sleep in hippocampus-dependent memory in rodents. Hippocampal "place cells" that were active during maze running were more likely to be activated during subsequent sleep, a phenomenon known as neural replay. In addition to hippocampal neural replay, it has been asserted that the hippocampus and cortex communicate during sleep by means of hippocampal generated high frequency burst patterns, which are temporally correlated with spindles in the medial prefrontal cortex during slow-wave sleep. Despite this evidence not a single study specifically demonstrates improved memory after sleep in rodents, although several studies demonstrate deficits in memory due to sleep deprivation.
The most commonly used approach to examine behavioral effects of sleep on memory consolidation has been the sleep deprivation method. Sleep deprivation studies typically find that handling the animals during the sleep phase (i.e. depriving sleep) leads to memory deficits. Although this may be suggestive that sleep is important for memory, there may be other explanations for the impairment, such as stress-related deficits from the constant handling. In Chapter 1, we reexamine the effects of sleep deprivation (i.e. gentle handling) on rodent Pavlovian fear conditioning. We found that the deprivation method itself (i.e., gentle handling) induced deficits independent of sleep suggesting that the sleep deprivation method might be a problematic way to examine the role of sleep in memory consolidation. In Chapter 2, we detailed a naturalistic method to examine whether Pavlovian fear conditioning is enhanced after a sleep phase, as compared with an equivalent passage of an awake phase. We found that sleep selectively enhanced hippocampus-dependent memory in mice. In Chapter 3, we investigated the relationship between pharmacologically induced sleep and Pavlovian fear conditioning. We found that therapeutic doses of zolpidem (0.01-0.5 mg/kg) did not affect memory acquisition or consolidation. Additionally, we found that 8 mg/kg of zolpidem induced deep sleep. Next, we explored the effects of pharmacologically induced deep sleep and memory consolidation. We found that a drug-induced “mouse nap,” an episode of deep sleep during the awake phase, selectively enhanced contextual memory.

Next, we wanted to expand our findings to human memory. In Chapter 4, we utilized a nap paradigm to examine how sleep affected memory compared to caffeine and an awake-placebo control. We found that sleep improved consolidation of verbal memory compared to both caffeine and the control. Lastly, in Chapter 5, we took advantage of the nap paradigm to study how sleep integrates new information with prior experiences. We found that REM sleep, compared to NREM sleep and quiet wake,
improved problem solving by assimilating new information with past experience to create a richer network for future use. Both the rodent and human evidence provide strong support for the critical role of sleep in stabilizing and reorganizing new information.
INTRODUCTION

Evidence for Sleep-Dependent Memory Consolidation in Humans and Mice

Classically, memory consolidation refers to the process by which new memories that are initially in a fragile state stabilize and reorganize into various brain structures. Consolidation has been dichotomized into at least two phases, cellular and systems consolidation. Cellular consolidation is the stabilization of information storage at local neuronal circuits in the hippocampus, and presumably in the cortex (Bailey, Bartsch, & Kandel, 1996). The binding of glutamate to NMDA receptors allows calcium to enter the postsynaptic cell, which engages a host of molecular events that leads to lasting alteration of synaptic proteins, as well as synaptic remodeling and growth. This type of consolidation is thought to occur within minutes to hours after training (Dudai, 2004). Systems consolidation refers to a protracted process, on the order of weeks to years, by which memories become less dependent on the hippocampus and more reliant on other structures (Dash, Hebert, & Runyan, 2004). This consolidation process is thought to reflect coordinated activity whereby fast-changing connections in the hippocampus initially subserve the memory, and over time entrain slow-changing connections in the neocortex, at which time the hippocampus is no longer necessary to maintain the memory (Squire & Alvarez, 1995). This reorganization may be mediated by reactivation of the network during offline periods, such as sleep (Frankland & Bontempi, 2005; Ji & Wilson, 2007; Squire & Alvarez, 1995). Sleep has mainly been proposed to play a role in systems consolidation, facilitating the communication between the hippocampus and cortex (Ji & Wilson, 2007; Siapas & Wilson, 1998).

Under what conditions do the mechanisms of consolidation become engaged? The theory that sleep and memory are intimately entwined dates back to Ebbinghaus,
the founder of experimental memory research. In his seminal work on forgetting, he found that retention of nonsense syllables was surprisingly high after a 24-hour interval. While Ebbinghaus did not directly relate the role of sleep with memory, this observation led Jenkins and Dallenbach (1924) to systematically examine the beneficial effect of sleep on memory retention. The investigators found that there was less forgetting of nonsense syllables after a period of sleep compared to wake and suggested that sleep may simply delay forgetting, as no new competing information is encoded during this state (Wixted, 2004).

Because sleep is not an undifferentiated state, one focus of this line of research has been to identify the specific stage of sleep that is important for consolidation. Sleep is divided into 5 stages. Stages 1 through 4 refer to progressively deeper levels of sleep, with stages 3 and 4 often being referred to as slow-wave sleep (SWS). Rapid eye movement (REM) sleep is a lighter stage of sleep, typically associated with vivid dreams. For hippocampus-dependent memory, the beneficial effects of sleep have been mostly associated with SWS (Ellenbogen, Payne, & Stickgold, 2006; Marshall & Born, 2007; Marshall, Molle, Hallschmid, & Born, 2004; Rasch, Buchel, Gais, & Born, 2007; Tucker et al., 2006), while for non-hippocampus-dependent memories, the beneficial effects of sleep are more often associated with REM sleep (Fischer, Hallschmid, Elsner, & Born, 2002; Mednick, Nakayama, & Stickgold, 2003; Plihal & Born, 1999).

Evidence for sleep-dependent memory consolidation in rodents

One leading candidate theory for how memories are consolidated during sleep comes from the rodent neural replay literature. In 1989, Pavlides and Winson showed what appeared to be a neurophysiological correlate of memory processing during sleep (Pavlides & Winson, 1989). Hippocampal “place cells” that were active during maze
running were more likely to be activated during subsequent sleep periods. Since then, similar results of neural replay during sleep have been observed (Qin, McNaughton, Skaggs, & Barnes, 1997; Wilson & McNaughton, 1994) and the temporal sequence of paired neuronal firing during wake has also been found to be preserved in subsequent non-REM sleep (Skaggs & McNaughton, 1996). In addition, there is evidence that multi-cell sequences replay in sleep and do so at an accelerated rate (i.e. time-compressed) (Hirase, Leinekugel, Czurko, Csicsvari, & Buzsaki, 2001; Kudrimoti, Barnes, & McNaughton, 1999). The neural replay that occurs during non-REM sleep occurs at a rate 5-10 times faster than it did during the waking state and REM (Ji & Wilson, 2007).

In addition to replay of neural patterns in the hippocampus, it has been asserted that the hippocampus and cortex communicate via neural replay during sleep, resulting in deeper encoding of the experience during wake. One candidate theory for how information from the hippocampus is transferred to the cortex is by high frequency burst patterns in the hippocampus during SWS, also known as sharp waves or ripples (Buzsaki, 1989), which have been shown to be temporally correlated with cortical spindle (Siapas & Wilson, 1998). Replay in the hippocampus and cortex of rats has been temporally correlated during slow-wave sleep (SWS), further suggesting hippocampal-cortico dialogue during sleep (Ji & Wilson, 2007). More recently, neural ensembles from the medial prefrontal cortex have been found to coactivate with hippocampal sharp wave/ripple complexes during SWS after learning a new rule (i.e. changing arms on a Y-maze; Peyrache, Khamassi, Benchenane, Wiener, & Battaglia, 2009). All this has been taken to suggest that hippocampal-dependent memories are stabilized and distributed (i.e., consolidated) to the cortex during sleep.
Neural replay has most often been observed in rats during slow wave sleep. It has also occasionally been observed during REM sleep, but unlike the compressed rate at which it occurs during SWS, the rate of replay during REM is similar to the neuron firing that occurred during learning (Louie & Wilson, 2001) and thus may simply reflect dreaming. It is as if the hippocampus is replaying the earlier behavioral experience, perhaps as a way to reorganize the representation of that experience in the cortex.

There is little doubt about the presence of neural replay, but the significance of replay is still questionable. First, neural replay is not specific to sleep. Correlated place cell activity has been detected during periods of active and quiet wake (Karlsson & Frank, 2009; Nakashiba, Buhl, McHugh, & Tonegawa, 2009; O'Neill, Senior, & Csicsvari, 2006). Second, replay is generally detected after saturation of learning and not during initial learning. Rodents are typically trained for several hours each day for weeks at a time before replay is detected. Thus, replay may not necessarily reflect the strengthening of a memory trace but rather a decaying resonance of a well-engrained pattern of neural activity. Lastly, if replay reflects learning, then it should predict performance on tasks. To date, no study has shown that an increase in replay results in better performance.

Animal studies linking sleep and behavioral performance have primarily used a sleep-deprivation paradigm. Earlier studies trained rodents on a task (usually during the light phase which also happens to be a nocturnal animal's sleep phase) and then sleep deprived the animals by placing them on a rotating platform over water. This method of sleep deprivation has been criticized for its potential stress inducing effects on memory (Horne & McGrath, 1984; Smith, 1985; Vertes & Eastman, 2000) and recent sleep deprivation studies have used a milder approach, mainly handling the animals during the
sleep phase (Graves, Heller, Pack, & Abel, 2003). These studies have shown that sleep deprivation following learning leads to poorer performance on retrieval tests than those that had normal sleep. While this may be suggestive that sleep is important for memory, there may be other causes for the impairment. Indeed, mild sleep deprivation has been shown to increase serum levels of glucocorticoids, which in turn been shown to negatively affect cognition (Plihal, Krug, Pietrowsky, Fehm, & Born, 1996). LTP response has been reported to decrease in hippocampal slice preparations from sleep-deprived rats compared with normal awake rats and this was correlated with increased corticosterone (Campbell, Guinan, & Horowitz, 2002). From the animal sleep-deprivation literature, it is unclear if the decrements are due to the lack of sleep or the methods used in depriving sleep.

In Chapter 1, we reexamined the effects of sleep deprivation on Pavlovian fear conditioning. In Pavlovian fear conditioning, a tone is paired with a shock in a distinct context. After a single pairing, rodents will exhibit fear when presented with the training tone, or when returned to the training environment. This latter phenomenon, known as contextual fear conditioning, has garnered considerable interest because it is hippocampus-dependent and has become a prominent rodent model of declarative memory (Anagnostaras, Gale, & Fanselow, 2001; Anagnostaras, Maren, & Fanselow, 1999). Pavlovian fear conditioning is well-suited for the examination of the role of sleep and memory because it is rapidly acquired and can dissociate between hippocampus-dependent and -independent memory. Consistent with human declarative memory, contextual fear gradually becomes independent of the hippocampus, as this memory is consolidated to neocortical structures (Maren, Aharonov, & Fanselow, 1997) (Anagnostaras et al., 1999; Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004; Quinn, Ma, Tinsley, Koch, & Fanselow, 2008). In contrast, tone (cued) fear is
independent of the hippocampus (Anagnostaras et al., 2001), yet both contextual and
cued fear memory depend on the amygdala for the animal’s lifetime (Gale et al., 2004).

In Chapter 1, animals were trained on fear conditioning during the hour prior to
their main sleep phase and tested 72 hours after training. During the first 24 hours
following training, mice were either gently handled during their sleep phase, awake
phase, or were left alone in their home cage. As expected, we found that handling mice
in their sleep phase impaired contextual memory compared to non-handled controls.
However, simply handling animals in their awake phase induced the same magnitude of
impairment compared to the sleep deprivation group. These results suggest that the
deprivation method itself (i.e., gentle handling) induced deficits independent of sleep.
These data indicate that sleep deprivation is a problematic way to examine the role of
sleep in memory consolidation, and an alternative paradigm is proposed.

In Chapter 2, we used a novel, naturalistic paradigm to study the effect of a sleep
phase on Pavlovian fear conditioning. Mice were trained 1 hour before their sleep/rest
phase or awake/active phase and then tested for contextual and cued fear 12 or 24 hr
later. We found that hippocampus-dependent contextual memory was enhanced if tested
after a sleep phase within 24 hr of training. This enhancement was specific to context
and not cued memory. These findings provide direct evidence of a role for sleep in
enhancing hippocampus-dependent memory consolidation in rodents and detail a novel
paradigm for examining sleep-induced memory effects.

Next, we wanted to further investigate the characteristics of sleep underlying
consolidation. While much of the replay literature suggests that SWS is important for
hippocampal memory consolidation, there has been no direct rodent evidence that this
deep sleep improves memory. In addition to correlating sleep stages with behavioral
performance, we are also experimentally manipulating sleep stages with pharmacology. In Chapter 3, we performed a dose-effect analysis on zolpidem (a hypnotic drug) and sleep, in which we found 8mg/kg of zolpidem to induce deep sleep in mice. Others have also reported that a similar dose (10mg/kg) resulted in a 20% increase of SWS in rodents (Depoortere et al., 1986). In a follow-up study, we trained animals on fear conditioning right before their main awake or sleep phase. Immediately following training, we administered 8mg/kg of zolpidem (i.e. induced deep sleep) or saline and tested them 12 hours later. Our results suggest that pharmacologically induced deep sleep selectively enhances consolidation of hippocampus-dependent memory, consistent with the neural replay literature.

**Evidence for sleep-dependent memory consolidation in humans**

In Chapters 4 and 5, we examined the role of sleep on human memory. Some difficulties that studies have to overcome is that circadian and homeostatic confounds are intrinsically intertwined with sleep and memory research. In nocturnal sleep studies that attempt to control for circadian factors, subjects typically learn a list of word-pairs in the evening prior to sleep or sleep-deprivation. Subjects are tested either the following morning (Ellenburg, Hulbert, Stickgold, Dinges, & Thompson-Schill, 2006) or after a night of recovery sleep (Hu, Stylos-Allan, & Walker, 2006). Subjects, who have slept, typically perform better compared to those who were sleep-deprived. While this may be suggestive that sleep is important for memory consolidation, the impairment may very well be caused by non-sleep related factors, as demonstrated in Chapter 1. Performance deficits might also be attributed to residual fatigue from sleep-deprivation, even after a full night of recovery sleep. Recent imaging studies have shown that it takes more than one night of recovery sleep for the human brain to return to use of it’s normal neural
network for a declarative task (McKenna et al., submitted). This suggests that sleep-deprivation may be a different brain state than wake, and it may impair normal consolidation.

To relieve sleep deprivation confounds, many researchers have turned to an alternative method of allowing normal sleep and normal wake periods to pass between training and test. Typically, the sleep group learns a list of words at night and is tested in the morning after a period of sleep (Ellenbogen, Hultbert et al., 2006). The wake group learns a list of words in the morning and is tested in the evening after an equivalent delay interval. Similar to the seminal findings by Jenkins and Dallenach (1924), recall is typically better after a night of sleep compared to a day of wake and researchers have used this result to argue the importance of sleep in consolidation. While this alternative method circumvents the sleep deprivation issue, there are still circadian and homeostatic concerns. Subjects may be more alert after a nights sleep and may be better able to retrieve the learned information than at the end of a busy day. In Chapter 4, we used a nap paradigm to investigate how sleep compared to caffeine (a popular stimulant used to increase alertness and arousal) and placebo on different memory domains, including declarative memory. By using a nap paradigm, we addressed circadian confounds by having the sleep and wake groups train and test at the same time. We additionally addressed circadian concerns by adding an extra bout of sleep during the day, a time which is not biologically tied to sleep. We control for homeostatic measures by having additional memory tests after the nap to distinguish between specific improvements for items learned prior to the nap and general improvement due to increased alertness or arousal after a nap. We found that naps benefited memory, including verbal retention, more than caffeine or placebo controls. This benefit was not a result of general increase in alertness or arousal but specific to the consolidation process.
Next we wanted to explore the role of REM sleep in memory consolidation. REM sleep has been often linked with the consolidation of implicit memories, such as visual and motor memories (Fischer et al., 2002; Mednick et al., 2003; Plihal & Born, 1999), which are dependent on neocortical structures. One plausible explanation is that REM sleep may be a brain state that facilitates the integration of cortical associations. REM sleep has often been the state associated with vivid dreaming and many have posited that this brain state facilitates the forming of combinations of ideas in new or useful ways (i.e. creativity). In Chapter 5, we asked how sleep would benefit the integration of new information with prior representations in humans. We found that REM sleep (compared to NREM sleep and quiet wake) improved problem solving by priming associative networks. These results suggest that REM sleep is important for assimilating new information with past experience to create a richer network for future use.
Brief Communication

Sleep deprivation and Pavlovian fear conditioning

Denise J. Cai,1,3 Tristan Shuman,1 Elizabeth M. Harrison,1 Jennifer R. Sage,1 and Stephan G. Anagnostaras1,2

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Sleep has been suggested to play a role in memory consolidation, although the exact nature of this relationship remains unclear. Human studies, sleep-deprived subjects typically have impaired memory retention (Bonnet and Arand 1995; Arnold et al. 1997; Carter et al. 2003). This impairment, however, may be caused by non-sleep factors associated with the method of deprivation, such as stress. Sleep deprivation in humans can cause physiological stress, marked by stomach ulcers and elevated cortisol (Mullington et al., 2009), that could interfere with memory (Sapolsky 2004). Moreover, the fatigue associated with sleep deprivation may produce performance deficits, even after a night of recovery sleep. One recent imaging study found that it takes more than one night of recovery sleep for the human brain to return to normal use of its neural network (McElneny et al. 2009). This suggests that sleep deprivation produces changes in brain state that could impair normal consolidation or retrieval.

To avoid these confound, many researchers have turned to a more naturalistic method of allowing normal sleeping or waking periods to pass between training and testing. Typically, a sleep group is trained at night and tested the morning after a period of sleep, while an awake group is trained in the morning and tested in the evening after an equivalent delay interval. Recall is often better after a night of sleep (Ellenberg et al. 2006; Nishida et al. 2009). This alternative method circumvents the confounds of sleep deprivation; however, most animal studies continue to use sleep deprivation to explore the effects of sleep on memory consolidation.

Early studies deprived animals of sleep by placing them on a rotating disk or on top of an inverted partially submerged flower pot over water (Dement et al. 1968; Van Hulsen and Coenen 1979; Murison et al. 1982; Rechtschaffen et al. 1989). These methods of sleep deprivation have been highly criticized for their potentially stress-inducing side-effects (Home and McGrath 1984; Smith 1985; Vertes and Eastman 2000). Recent sleep-deprivation studies have utilized a gentler approach—handling the animals during sleep. However, even this handling method has been shown to reduce toms effects (Murison et al. 1982) and increased levels of glucocorticoids, which can impair cognition (Pittal et al. 1996; Sapolsky 2004). In fact, long-term potentiation is diminished in area CA1 from sleep-deprived rats, and this correlates with increased corticosterone levels (Campbell et al. 2002). Finally, because sleep is a homeostatic drive, the buildup of sleep debt is likely stressful in itself (Roehrs et al. 1996; Carter et al. 2003; Anderson and Home 2009). Thus, while the rodent sleep deprivation literature suggests that sleep is important for memory consolidation, it is unclear whether these effects are caused by the absence of sleep or by non-sleep-related consequences of deprivation.

An ideal task to examine the relationship between sleep and memory consolidation in Pavlovian fear conditioning, in which a tone is paired with a shock in a distinct context. After a single pairing, rodents will exhibit fear when presented with the training tone, or when they returned to the training environment. This latter phenomenon, known as contextual fear conditioning, has garnered considerable interest because it is hippocampus-dependent and has become a prominent model of declarative memory (Anagnostaras et al. 1999, 2000, 2001, 2002a). Pavlovian fear conditioning is well-suited for the examination of the role of sleep and memory because it is rapidly acquired and can dissociate between hippocampus-dependent and independent memory. Consistent with human declarative memory, contextual fear gradually becomes independent of the hippocampus, as this memory is consolidated to neocortical structures (Maren et al. 1998, Anagnostaras et al. 1999; Frankland et al. 2004; Quinn et al. 2008). This consolidation process is thought to reflect coordinated activity whereby fast-changing connections in the hippocampus initially subserve the memory, and over time entrain slow-changing connections in the neocortex, at which time the hippocampus is no longer necessary to maintain the memory (O’Mara and Alvarez 1995). In contrast, tone (cued) fear is independent of the hippocampus (Anagnostaras et al. 2001), yet both contextual and cued fear memory depend on the amygdala for the animal’s lifetime (Gale et al. 2004).

Graves and colleagues examined the effects of sleep deprivation on Pavlovian fear conditioning (Graves et al. 2003). They found that sleep deprivation impaired performance only when administered immediately following training. Several issues make their data difficult to interpret. First, all three groups (non-sleep-deprived, sleep deprived immediately after training, and sleep deprived 5 h after training) were trained and tested in the main sleep period (Fig. 1A). Training the animals during their main sleep period likely induced sleep deprivation in all groups, including the Non-Sleep-Deprived group. Moreover, testing mice during this phase is equally problematic (Chaudhary and Cobell 2002). Second, the animals were tested within 24 h of the sleep deprivation.
and performance deficits could result from residual fatigue (Rickard et al. 2008; McKenna et al. 2009). Third, there was no adequate control for the sleep deprivation method used, to demonstrate that it was lack of sleep per se that produced the deficit. Therefore, it is still unclear whether the impairments observed with sleep deprivation are due to the lack of sleep or other non-sleep-related effects.

In experiment 1, we expanded on the findings of Graves et al. (2003) and used a design that addresses the confounds listed above, to reexamine how sleep deprivation by gentle handling, and the handling manipulation itself, affects memory consolidation. In experiment 2, we examined an alternative method of sleep deprivation by administering two spaced injections of a moderately high dose of amphetamine.

Subjects
Sixty-seven (Exp 1) and 65 (Exp 2) hybrid C57BL/6J x 129F1/SvEms/J (Clavley et al. 1997; Mynatia et al. 2008) male and female mice (Jackson Laboratories, Bar Harbor, ME) were balanced across groups. Mice were entrained to a 12:12 light/dark cycle. All animals were handled five times for 1 min each in both the dark and light phases.

Experiment I
Pavlovian fear conditioning was conducted 1 h before the primary sleep period (Fig. 1B). Mice were placed in a fear conditioning chamber (see Conditioning Contexts in Cai et al. 2009; Wood and Akanagnostaras 2009) and, after a 2-min baseline, received one tone (2.8-kHz, 30-s, 85-dB) footshock (3-s, 1.0-mA, SC) pairing; they remained there for an additional 5 min extended post-shock freezing test. Mice then received one of three treatments. The Control group was undisturbed in their home cage until testing 72 h later. The Sleep/Handle group was sleep deprived throughout the entire subsequent sleep phase, by gentle handling. Specifically, experimenters continuously observed the animals (with dim red head-mounted LED lights) and gently prodded the mouse whenever it was falling asleep. Sleep was defined as a lack of motion (except that required for respiration) and the eyes closed. To control for non-sleep-related effects from handling, the Awake/Handle group was handled throughout the entire subsequent awake period, beginning 12 h after training. This group was gently handled once every 15 min during their awake phase. Freezing was measured according to an automated algorithm (Wood and Akanagnostaras 2009). A one-way ANOVA revealed no group differences during training (F<sub>2,64</sub> = 1.0, n.s.), and all groups exhibited significant learning (F<sub>exp</sub> = 17.5, P < 0.001, Fig. 2A).

Contextual fear was examined 72 h after training by returning them to the conditioning chamber for 5 min (Fig. 2B). Handling during either the awake (Awake/Handle) or sleep phase (Sleep/Handle) impaired contextual fear memory compared with controls. A univariate ANOVA found group differences during the context test (F<sub>2,64</sub> = 4.24, P < 0.02), and post-hoc analysis using Fisher's LSD revealed significant differences (P < 0.05) between the Control group and both Handle groups, which did not differ from one another (P > 0.8, n.s.).

Thirty minutes following the context test, mice were placed in a novel context and given a 5-min cued fear test. After a 2-min baseline period, the training tone was presented three times without shock. Mice that were handled during the sleep phase (Sleep/Handle) were significantly impaired compared with Control mice, while mice that were handled during the awake phase...
Sleep deprivation and memory

Figure 2. (A) Training. After a 2-min habituation period, mice were given one tone-shock pairing and were left in the chamber for a total of 10 min. Freezing (mean percent time ± SEM) is depicted for each minute of the training session. All groups exhibited robust learning, and there were no group differences. (B) Contextual Fear. Seventy-two hours post-training, mice were given a 5-min contextual fear test, and freezing (mean percent time ± SEM) for the 5-min test is depicted. Both the Awake/Handle and Sleep/Handle groups are compared with the Control group. (C) Cued Fear. Thirty minutes after the context test, mice were brought to a novel context for a 1-min cued fear test. After a 2-min baseline period, the testing tone was presented three times across 1 min. Cued fear is depicted as freezing (mean percent time ± SEM) during the baseline subtracted from the average freezing to the three tones. The Sleep/Handle group had a deficit compared with the Control group, while there were no differences between Awake/Handle and Control groups.

(Awake/Handle) showed no deficit (Fig. 2C). Freezing during the 2-min baseline was subtracted from the average freezing to the three tones. A one-way ANOVA revealed group differences ($F_{(3,64)} = 3.97$, $P < 0.02$), and post-hoc analysis using Fisher’s PLSD revealed a significant difference ($P < 0.05$) between the Control group and the Sleep/Handle group. No difference was found between the Control group and the Awake/Handle group ($P > 0.8$, n.s.). Overall, handling during the sleep phase produced pervasive deficits in contextual and tone fear. On the other hand, handling during the awake phase produced selective deficits in contextual fear.

Experiment 2

Training was identical to Exp 1, although a 4-min baseline period was used. After training, mice were given two injections of 8 mg/kg D-amphetamine hemisulfate (Sigma-Aldrich), i.p., or 10 mL/kg saline solution (Fig. 3C). The Sleep/Amph sleep-deprived group received amphetamine immediately after training, at the start of their main sleep phase, and 4 h later. The Sleep/Sal control group received saline at the same times. To control for effects of amphetamine, the Awake/Amph and Awake/Sal groups were administered amphetamine and saline, respectively, at the start of the main wake phase and 4 h later. A two-way ANOVA revealed no group differences during training ($F_{(3,60)} < 1$, n.s.), and all groups exhibited significant learning ($F_{(1,60)} = 17.5$, $P < 0.001$, Fig. 3A).

Contextual fear was examined 72 h after training (as in Exp 1). Amphetamine failed to produce any deficit in contextual fear conditioning when given during either the sleep or awake phase (Fig. 3B). A two-way ANOVA revealed no main effect of drug ($F_{(1,60)} < 1$, n.s.) or phase ($F_{(1,60)} < 1$, n.s.).

As in Exp 1, a tone test was conducted 30 min after the context test. Amphetamine failed to produce any deficit in cued fear conditioning when given during the sleep or awake phase (Fig. 3C). Freezing during the 2-min baseline was subtracted from the average freezing to the three tones. A two-way ANOVA revealed no main effect of drug ($F_{(1,46)} < 1$, n.s.) or phase ($F_{(1,46)} = 3.09$, n.s.). Overall, sleep deprivation by amphetamine had no effect on contextual or cued fear memory.

We found that gentle post-training handling impaired context memory regardless of sleep. These findings indicate that gentle handling produces contextual memory deficits through non-sleep-related factors. Moreover, handling during the sleep phase disrupted hippocampal-striatal-dependent memory. This is in contrast to Graves and colleagues (2003), who found that sleep-deprivation administered immediately after training only impaired contextual fear, while cued fear was spared. These discrepancies may be due to procedural differences. Graves et al. trained and tested all groups in the middle of the day, which is the main sleep phase for mice (Fig. 3A). Mice were then given no handling ("non-sleep-deprived") or 5 h of handling either immediately after training ("0–5 h") or 5 h later ("5–10 h"). This protocol raises a number of issues. First, all mice experienced some sleep deprivation because sleep was interrupted to give training and testing. Therefore, any sleep deprivation effect observed is additive with a sleep deprivation baseline. Second, deprivation in the 0–5 h group did not span the entire sleep period, nor did it target a specific sleep period, since handling was given 5 h after the sleep phase began. Third, the 5–10 h group did not target sleep or awake periods specifically, as handling overlapped both phases. Fourth, the study did not have a proper control group to examine the effects of handling alone on the memory impairment (i.e., handling only in the awake phase). Due to these confounds, the results of the Graves et al. study are problematic.

Our findings suggest that gentle handling is not a selective method of sleep deprivation. First, extensive handling during the entire sleep phase appears to produce non-sleep-related memory impairment. Second, with regard to contextual memory, handling during the awake phase can induce the same magnitude of amnesia as sleep deprivation. This suggests that there are non-sleep-related consequences of extensive handling (e.g., stress, interference, etc.) that may impair memory. This interpretation is consistent with...
prior critiques of sleep deprivation studies, which found that seemingly benign handling methods can induce health problems and an elevated stress response (Murison et al. 1982; Mullington et al. 2009). Thus, this common method of sleep deprivation is confounded by non-sleep-related effects and is not an ideal tool for investigating the relationship between sleep and memory.

To explore an alternative to handling, we examined pharmacological sleep deprivation. We administered two high doses (8 mg/kg) of amphetamine immediately after training and 4 h into the sleep phase, and found that this method failed to produce impairments of contextual or cued fear. One possibility for the lack of impairment is that the drug did not actually depabbrev the animals. This is unlikely, as this high dose produces insomnia and hyperlocomotion (Erwin et al. 1981). Anagnostaras and Robinson 1996; Anagnostaras et al. 2003b; Wood and Anagnostaras 2009) and D-amphetamine has a long, although variable, half-life (3.5–4.2 h in acute urine (Bufton 2006). Another possibility is that amphetamine had a positive effect on consolidation that counteracted the deprivation impairment. This also seems unlikely, as we have found pervasive memory deficits using this dose of amphetamine (alone) during training (Wood and Anagnostaras 2009). Alternatively, these data could indicate that sleep is not involved in memory consolidation. In this manner, stimulant-induced sleep deprivation may be unique in that it does not produce an amnesic stress response. Nonetheless, the unknown effects of amphetamine on stress and consolidation suggest that stimulants are not a viable method to investigate the relationship between sleep and memory.

Sleep deprivation itself may be inherently stressful, perhaps due to a build up of a sleep homeostatic drive. Because sleep is typically treated as a drive (need), governed by homeostatic factors, a “sleep pressure” is thought to build up, which is suggested to be aversive and stressful (Bolles 1967). If this is the case, no sleep-deprivation method will satisfactorily dissociate the effects of stress and lack of sleep on cognitive impairment. Therefore, we favor using a more naturalistic paradigm to investigate the consolidation process that may occur during natural sleep. In a separate study published elsewhere, we explored this using a novel rotodent paradigm (Cai et al. 2009). We compared 12- and 24-h delay intervals to control for pre-sleep and circadian effects. We controlled for differences in circadian activity by selecting training and testing times within the transition interval from night to day, respectively. That study found that sleep plays an important and selective role in contextual fear conditioning, whereas contextual memory is enhanced only when tested after a sleep period. This approach provides a useful way to examine sleep-induced memory enhancement, while avoiding confounds associated with the sleep-deprivation paradigm.

The current data suggest that it is problematic to use sleep deprivation as a way of exploring the role of natural sleep in memory consolidation. Although gentle handling produced amnesia, it was not specific to the sleep phase, making it difficult to disentangle the cause for the memory impairments. Nonetheless, the effects of sleep deprivation may be intrinsically interesting, as sleep deprivation is so prevalent in modern society (Kronert and Azard 1995; Arnold et al. 1997; Anderson and Home 2008). However, rather than extensive handling of animals, we would encourage utilization of more naturalistic modeling of sleep deprivation.

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References
Mullington JM, Haack M, Toth M, Serrador JM, Meier-Ewert HK. 2009.


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Chapter 1, in full, is a reprint of the material as it appears in Sleep-deprivation and Pavlovian fear conditioning. *Learning & Memory.* 16(10), 595-599. Cai, D.J., Shuman, T., Harrison, E.M., Sage, J.R., & Anagnostaras, S.G. (2009). The dissertation author was the primary investigator and author of this paper.
A growing body of evidence suggests that sleep enhances consolidation of human memory. Several studies have found that sleep facilitates retention of declarative memory (Ellenbogen, Hulbert, Stickgold, Dinglas, & Thompson-Schill, 2006; Ellenbogen, Payne, & Stickgold, 2006; Pihl & Born, 1999), and recently formed episodic memories can reportedly even be cued by an odor present during training to increase memory reactivation during slow-wave sleep (SWS) and improve subsequent memory retention (Rasch, Buchel, Gais, & Born, 2007). In one view, sleep-related memory reactivation may be the consolidation mechanism by which declarative memory is transformed from a hippocampus-dependent state to an independent state (Pavlovs & Winson, 1989; Poe, Nitz, McNaughton, & Barnes, 2000; Squire & Alvarez, 1995). Here, we demonstrate, for the first time, sleep-related improvements specific to hippocampus-dependent memory for a rodent learning task with a well-defined neurobiology—Pavlovian fear conditioning (Anagnostaras, Gale, & Fanselow, 2001; Anagnostaras, Marek, & Fanselow, 1999; Gale et al., 2004).

In Pavlovian fear conditioning, a tone is paired with a shock in a novel environmental context. After training, rodents will exhibit fear when presented with the training tone or when returned to the training environment. This latter phenomenon, known as contextual fear conditioning, has garnered considerable interest in recent years because it is hippocampus dependent and has become a prominent rodent model of declarative memory (Anagnostaras et al., 2001). As with human declarative memory, over time contextual fear becomes independent of the hippocampus, as this memory becomes consolidated to neocortical structures (Anagnostaras et al., 1999; Frankland, Bontemps, Talfon, Kazemzadeh, & Silva, 2004; Marek, Abarsonov, & Fanselow, 1997; Quinn, Ma, Tinsley, Koch, & Fanselow, 2008). This consolidation process is thought to reflect coordinated activity whereby fast-changing connections in the hippocampus initially subserve the memory and, over time, entrain slow-changing connections in the neocortex, at which time the hippocampus is no longer necessary to maintain the memory (Squire & Alvarez, 1995). The memory may also change in content during this period so that small bits of episodic memory become integrated into cohesive and permanent semantic knowledge (McClelland, McNaughton, & O’Reilly, 1995). In contrast, tone (cued) fear is independent of the hippocampus (Anagnostaras et al., 2001; Anagnostaras et al., 1999). Both contextual fear memory and cued fear memory depend on the amygdala for the lifetime of the rat (Gale et al., 2004).

Considerable indirect evidence suggests a role for sleep in hippocampus-dependent memory in rodents. In 1989, Pavlovs and Winson (Pavlovs & Winson, 1989) showed what appeared to be a neurophysiological correlate of memory processing during sleep. Hippocampal “place cells” that were active during maze running were more likely to be activated during subsequent REM and non-REM sleep, a phenomenon known as neuronal replay. Since then, similar results of replay during sleep have been observed (Qui, McNaughton, Skaggs, & Barnes, 1997; Wilson & McNaughton, 1994), and the temporal sequence of paired neuronal firing during wakefulness, arguably a neuronal trace of spatial memory, has also been found to be preserved in subsequent non-REM sleep (Skaggs & McNaughton, 1996). In addition to hippocampal neuronal replay, it has been asserted that the hippocampus and neocortex communicate during sleep by means of hippocampal-generated high frequency burst patterns (sharp waves or ripples;
Bursaci, 1989), which are temporally correlated with spindles in
the medial prefrontal cortex during SWS (Siapas & Wilson, 1998).
This is especially significant for the present study, as the hip-
 pocampus is the initial site of acquisition for contextual fear
conditioning, whereas the medial prefrontal cortex is one site of
permanent memory storage (Frankland et al., 2004; Quinn et al.,
2000). Despite this evidence, to our knowledge, not a single study
specifically demonstrates improved memory after sleep in rodents
(Frank, 2000; Frank & Benington, 2006), although several studies
demonstrate deficits in memory due to sleep deprivation or posi-
tive correlations between the intensity of sleep components and
memory retention (Dutta, 2000; C. Smith & Butler, 1982).
The most commonly used approach to examine behavioral ef-
fects of sleep on memory consolidation has been the sleep depre-
vation method. Earlier studies trained rodents on particular tasks
and then deprived them of sleep by placing them on a rotating disk or
on top of an inverted partially submerged flower pot over water
(Bergman et al., 1989; Poulton, 1971; Murison, Ursin, Cooper,
Lett, & Ursin, 1982; Van Hulzen & Coenen, 1979). These meth-
ods of sleep deprivation have been criticized for their potentially
stress-inducing effects (for reviews, see Horne & McGrath, 1984;
Smith, 1985; Vertes & Eastman, 2000). Recent sleep deprivation
studies have utilized an alternative, more benign approach, han-
dling the subjects during the sleep phase; for example, Gravels
and colleagues (Graveley, Hand, & Abel, 2003) found that mice
that were handled for 5 hr during the sleep/ light phase exhibited
some impairments in contextual fear conditioning. Although this
may be suggestive that sleep is important for memory, there may
be other explanations for the impairment. Indeed, even the more
benign sleep deprivation method has been shown to induce health
problems, such as stomach ulcerations (Murison et al., 1982) and
increased serum levels of glucocorticoids, which in turn have
been shown to negatively affect cognition (Pihal, Kings, Pi-
terowski, Fehm, & Born, 1996). It has been reported that long-
term potentiation (LTP) is diminished in hippocampal slice pre-
parations from sleep-deprived rats and correlates with increased
corticosterone levels (Campbell, Guinea, & Honorowicz, 2002).
Thus, although the rodent sleep deprivation literature is suggestive
that sleep is important for memory consolidation, it is still unclear
whether the crucial component is the lack of sleep or the depriva-
tion methods itself that impairs consolidation.
The present study used a naturalistic method to examine
whether Pavlovian fear conditioning is enhanced after a sleep/ light
phase, as compared with an equivalent passage of awake/ active
time. In the fear conditioning literature, investigators often find
lower levels of contextual memory 30 min after training, with an
enhancement 24 hr after training that persists for long periods of
time (Miller et al., 2002). We asked if sleep might play a role in
this enhancement. We used 12- and 24-hr intervals to control for
time passage and circadian effects, adapted from designs used to
investigate human sleep and memory consolidation (Walker,
Braekefeld, Morgan, Hibbon, & Stickgold, 2002). It is critical to
properly control for passage of time, as consolidation may simply
be a time-dependent process. We also controlled for differences in
circadian activity by selecting training and testing times within the
transition intervals between rest and activity phases (Chaudhury &
Colwell, 2002). Our findings suggest that sleep plays an important
and selective role in contextual fear conditioning, whereby con-
textual memory was enhanced if tested after a sleep period. These
findings provide compelling evidence of a role for sleep in
hippocampus-dependent memory.

Method

Subjects and Materials
Six- to seven-hybrid C3HBl6/Alc;297TsvsJemail (Crawley et al.,
1997) male and female mice (approximately equivalent numbers
of sexes, stock from the Jackson Laboratory, West Sacramento,
CA) were balanced across groups. Mice were entrained to a 12-hr
light/dark cycle 5 weeks before the experiment began and re-
mained in those conditions for the duration of the experiment.
Lights were automated to turn on at 9 a.m. and turn off at 9 p.m.
They had unrestricted access to food and water. All experiments
were conducted in accordance with the National Institutes of
Health Guide for the Care and Use of Laboratory Animals and
were approved by the University of California, San Diego, Insti-
tutional Animal Care and Use Committee.

Fear Conditioning
Mice were trained 1 hr before their primary awake period (i.e.,
dark phase) for the Awake First conditions or were trained 1 hr
before their main sleep period (i.e., light phase) for the Sleep First
conditions. For the Awake First groups, the dark phase immedi-
ately followed training for a duration of 12 hr, in which then began
the light phase, also for a duration of 12 hr. For the Sleep First
group, the reverse occurred. The light phase began immediately
after training for 12 hr, followed by 12 hr of darkness. In both
groups, mice were tested 12 or 24 hr later for contextual and tone
fear memories (see Figure 1). During training, mice were placed in
a fear conditioning chamber (see Conditioning Context) and, after
a 2-min baseline, were given three tone–footshock (tone: 2.8 kHz,
30-s, 85-dB; footshock: 2-s, 1.0 mA) pairings, each 1 min apart.
After an additional 5 min, which served as an extended postshock
freezing test, they were returned to their home cages. To test
contextual memory, we placed the mice back in the original
chamber (12 or 24 hr postraining) for 2 min (Anagnostaras,
Maren, Sage, Goodrich, & Fanselow, 1999). Mice were also tested
for cued memory 1 hr later; they were placed in an alternate
context (discussed later) for a 2-min baseline period, followed by
presentation of the same three successive 30-s tones played during
training, each separated by 30 s, now without shock. Freezing and
gross movement were assessed for the entire training and testing
periods using an automated algorithm (discussed later).

Figure 1. Study timeline. Mice were trained 1 hr before their awake/active phase (Awake First) or before their sleep phase (Sleep First) and then tested for contextual and cued fear 12 or 24 hr later.
Conditioning Context

Four mice were tested concurrently in individual conditioning chambers housed in a windowless room. Each chamber (32 cm wide; 25 cm high, 25 cm deep) was located within a sound-attenuating chamber (65.5 cm wide, 35.5 cm high, 76 cm deep; Med-Associates Inc., Georgia, VT) and equipped with a speaker in the side wall, a stainless steel grid floor (36 rods, with each rod 2 mm in diameter and 8 mm center to center; Med-Associates Inc., Georgia, VT), and stainless steel drop pan. During each trial, chambers were scented with 7% isopropyl alcohol to provide a background odor, and background noise (65 dB) was provided by internal fans. Each sound-attenuating chamber was equipped with an overhead LED light source providing white and near-infrared light and an IEEE 1394 progressive scan video camera with a visible light filter (VID-CAM-MONO-2A; Med-Associates Inc., Georgia, VT) connected to a computer and video equipment in an adjacent room. Each chamber was connected to a solid-state scrambler providing AC constant current shock, and an audio stimulus generator controlled through an interface connected to a Windows computer running Video Freeze (Med-Associates Inc., Georgia, VT), a novel program designed for the automated assessment of freezing and motor activity. In results that will be published more fully elsewhere, computer- and human-scored data had a correlation of .971 and a linear fit of computer = −0.007 + 0.974 x human (for additional details, see, for e.g., Anagnostaras, Joselyn, Frankland, & Silva, 2000; Shuman, Wood, & Anagnostaras, in press; Wood & Anagnostaras, 2008). Motor activity scores correspond roughly to the number of video pixels changing per second at 30 Hz; for this reason, they are presented as arbitrary units (au).

Alternate Context

For testing cue (tone) fear, the conditioning context was modified along several dimensions. White acrylic sheets were placed over the grid floor to provide a different sensory experience, and a black plastic, triangular tent translocated only to near-infrared light was placed inside each box, with each side of the triangle measuring 23 cm. Only near-infrared light was used, creating a completely dark environment visible only to the video camera. Between tests, the chambers were cleaned and scented with a 5% white vinegar solution.

Statistics

Data were entered into a general multivariate analysis of variance (MANOVA). After an omnibus comparison, we made group comparisons using the Wald test. The level of significance was set at $p < .05$.

Results

Mice were given fear conditioning either right before their awake/active (Awake First) phase or right before their sleepiest phase (Sleep First) for the first day. Although mice sleep in both the light and dark phases, they spend much more time sleeping in the light phase (48%) than in the dark phase (35%; Welsh, Richardson, & Dement, 1988). Data from the training session are shown in Figure 2. After a 2-min habituation period, mice were given 3 tone–shock pairings one min apart and left in the chambers for an additional 5 min as an extended postshock freezing period. All groups exhibited good acquisition, and there were no group differences in locomotor activity or shock reactivity due to time of day, as examined computer-scored movement at 30 Hz during the 2-min baseline before any shock and during the first 2-s shock on the training day. Computer-scored movement (mean arbitrary units plus or minus standard error of the mean) is depicted. All groups exhibited robust shock reactivity, and there were no group differences in terms of baseline (closed bars) or shock-elicited (open bars) activity.
with the baseline ($F$s > 9, $p$s < .0001), and there were no group differences in terms of baseline or shock-elicited activity ($F$s < 2.1, $n$s). Thus, differences in fear conditioning could not be attrib-
uted to differences in locomotor activity or in shock reactivity.

Twelve or 24 hr after training, mice were given a 2-min contextual fear test followed immediately by a 5-min cued fear test (12 hr posttraining: Awake First, $n$ = 16; Sleep First, $n$ = 20; 24 hr posttraining: Awake First, $n$ = 16; Sleep First, $n$ = 15; see Figure 3A). An overall multivariate analysis of variance (MANOVA; four levels of group, with context and tone tests) revealed a Group \times Test Type interaction, $F(6, 378)$ = 2.26, $p$ < .05; so planned comparisons were made. For the contextual fear test, mice in the Awake First group failed to exhibit robust memory when tested 12 hr later, compared with those in the Sleep First group, who were also tested 12 hr later but had robust memory, $F(1, 14) = 4.28$, $p$ < .05; or when compared with those in the Awake First group who still had the opportunity to sleep by being tested 24 hr later, $F(1, 16) = 8.63$, $p$ = .01. That is, a sleep phase
enhanced contextual memory, and this effect was not due simply
to passage of time. The Awake First and Sleep First groups tested
24 hr later did not differ from each other or from the Sleep First
group tested 12 hr later ($F$s < 1, $n$s). One hour after the context
test, mice were brought to a novel context for a 5-min cued fear
test. After a 2-min baseline period, the training tone was presented three times across 3 min. All groups exhibited robust tone-elicited freezing, and there were no group differences during the baseline or presentation of the three tones (for the baseline, or average of the three tones, or baseline subtracted from tone, all comparisons, $F$s < 1, $n$s). Cued fear is depicted in Figure 3B as freezing during baseline subtracted from the average of freezing to the three tones. Overall, the design revealed a remarkably selective effect of the
passage of a sleep period; contextual fear was impaired if tested
before a sleep period and enhanced after a sleep period, but cued
fear was unaffected by sleep.

Discussion

We found that the passage of a sleep/rest phase enhanced
contextual fear memory regardless of whether the sleep phase
occurred immediately after training or 12 hr later. This enhance-
ment was specific to context memory, as there were no differences
between groups for cued memory. It is important to note that the
group differences in freezing during the context test cannot be
explained by circadian variability, as there were no group freezing
differences for the cued test, which occurred at approximately the
same time. This suggests that the contextual memory deficit in the
12-hr Awake First group was not due to the inability to express this
memory. This group also controls for sleep, which may have
occurred during the awake/active phase (Welsh et al., 1988).
Despite 35% of sleep occurring during the dark (active/awake)
phase, this sleep was not sufficient to enhance contextual memory,
because the 12-hr Awake First group had very poor contextual
memory. This suggests that whatever sleep that did occur in the
dark phase was insufficient for consolidation. Although this could
simply be due to the lower quantity of sleep during the dark phase,
there may also have been a difference in the quality of sleep
(Welsh et al., 1988). Taken together, our findings also suggest a
more active role for sleep in consolidation. If consolidation relied
only on the passage of time, then the 24-hr groups would outper-
form the 12-hr groups, regardless of sleep. As observed, this was
not the case. The groups that had a sleep phase outperformed the
group that had only an awake phase, regardless of the retention
interval, suggesting that consolidation is not strictly time depen-
dent.

One surprising finding was that contextual fear conditioning
apparently decreased from training to 12 hr and then increased by
24 hr after training. One of our original motivations for the study
was anecdotal evidence that studies examining short-term memory
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(e.g., 30 min) often found fear levels lower than those found in studies of long-term memory (e.g., 24 hr; Miller et al., 2002). A review of the literature, however, suggests a longer history to this finding in aversive conditioning. In 1957, Leon Kamin published an influential paper in which he reported that weakly acquired avoidance fear conditioning dramatically decreased from an initial level at 1 hr after training and then increased at 24 hr after training (and continued to increase when tested at 19 days; Kamin, 1957). This finding came to be known at the time as the Kamin effect, which is now readily confused with the better known blocking effect. This retention effect motivated considerable research (for a review, see Brush, 1971) but over time was largely forgotten. Here we suggest that this Kamin retention effect was at least partially due to sleep.

Until now, most rodent behavioral studies examining the role of sleep in memory have utilized potentially stress-inducing sleep deprivation techniques (Home & McRath, 1984; Rechtschaffen, Gilliland, Bergmann, & Winter, 1983; C. Smith, 1985, Smith, Casway, & Rose, 1998; Vertes & Eastman, 2000; Youngblood, Zhou, Smagin, Ryan, & Harris, 1997). Recent sleep deprivation studies have used gentle handling of the animals during the sleep/rest phase (Graves et al., 2003; Munson et al., 1982) and have shown that sleep deprivation after learning leads to poorer performance on retrieval tests. However, even this seemingly more benign deprivation technique has been shown to increase serum levels of glucocorticoids, which in turn have been shown to negatively affect cognition and the hippocampus (Phihal et al., 1996; Sapolisky, 2004). Therefore, sleep deprivation is a problematic approach to understanding the role of naturally occurring sleep in memory consolidation processes. Our approach is more naturalistic, allowing for the passage of awake/active and sleep/rest periods. Although considerable sleep does occur during the awake/active phase for mice (Welsh, Richardson, & Dement, 1988), this was clearly not sufficient for good contextual memory in our study, as the 12-hr Awake First group had very poor memory compared with all other groups, which had the passage of a sleep/rest period.

In any study of sleep-related memory enhancement, three very significant confounds exist that must have adequate controls. First, sleep necessarily involves the passage of time, and therefore studies must show that an equivalent passage of time without sleep is not sufficient for the consolidation effect. In our study, we compared mice with and without sleep 12 hr after training and found that a sleep/rest period was necessary to show good contextual memory. Moreover, if mice in the Awake First group were allowed a sleep/rest period, by testing them 24 hr after training, they showed robust memory. Second, one must control for time-of-day effects, both in terms of training and testing. Circadian effects could pose a significant problem for fear conditioning studies, because the form of the fear response, locomotor activity, pain sensitivity, and memory retrieval could be sensitive to time-of-day effects (Chaudhury & Colwell, 2002; Eckel-Mahan et al., 2008). We used several strategies to avoid circadian confounds. We tested mice at 12 or 24 hr after training, and with a 12 hr light-dark cycle, this meant that the 12 hr time point was at the same time of day as the 24-hr time point for some groups (see Figure 1). That is, time of day was not the cause of poor memory in the 12-hr Awake First group, as the 24-hr Sleep First group was tested at the same time of day but showed robust memory. Moreover, we minimized time-of-day differences by training and testing mice at times close to the light–dark transitions when behavioral differences are smallest (Chaudhury & Colwell, 2002). This is evidenced by the fact that Awake First and Sleep First groups did not differ in training in terms of postshock freezing, shock reactivity, or locomotor activity (see Figure 2). Third, one should control for fatigue—in our case, how long the mouse had been awake before testing (Mednick, Nakayama, & Stickgold, 2003; Ruckard, Cai, Rieth, Jones, & Auk, 2008). Fatigue was not the cause of group differences described here, because the 12-hr Awake First group was awake no longer than the 24-hr Sleep First group, yet the latter had much better memory. Taken together, these findings do not seem to be attributable to simple passage of time, time-of-day effects, or fatigue. We believe that the present design may serve as a simple and efficient paradigm for avoiding these confounds in future sleep studies.

Sleep, therefore, plays an important role in the stabilization of contextual memory. For Pavlovian fear conditioning, this effect was specific to hippocampus-dependent memory, as cued memory was entirely unaffected by the presence or absence of an intervening sleep period. Thus, sleep may play an important role in consolidating memory as it moves from a hippocampus-dependent to neocortical state. Considerable indirect evidence suggests that replay of recent memories in the hippocampus may serve to entrain slow-changing connections in the neocortex during sleep (Pax, Nitz, McNaughton, & Barnes, 2000; Squire & Alvarez, 1995). One candidate theory for how this transfer occurs is through coordination of hippocampal sharp waves (ripples) and cortical spindles during SWR (Buzsaki, 1996; J & Wilson, 2007; Siapas & Wilson, 1998). These findings suggest that hippocampal-dependent memories may be strengthened and transferred (i.e., consolidated) to the neocortex during sleep. Alternatively, sleep may play a separate role in memory consolidation than what is meant by cellular or systems consolidation. Because the role of sleep has generally been identified in the range of days, it may be that sleep is involved in an intermediate form of consolidation between cellular and systems consolidation (McGaugh, 2000). Although interesting theoretically, it is too early to fully explain where sleep-related memory consolidation lies with respect to better studied forms of consolidation. Rather, we favor the interpretation that the first day of consolidation, as examined in the present study, represents the beginning of systems consolidation.

Why, then, does the brain need a sleep phase to consolidate memory? By one view, the hippocampus may need to switch from an acquisition or an “in-flow” mode to a consolidation or “out-flow” mode to accomplish the transfer of memories to the neocortex. Acetylcholine has been suggested as one mechanism for switching the hippocampus from acquisition during wakefulness to consolidation during sleep (Anagnostaras, Maren, & Fanselow, 1995, 1999; Anagnostaras, Maren, Sage, Goodrich, & Fanselow, 1999, 1995, 1999; Anagnostaras et al., 2003, Buzsaki, 1989; Hasselmo, 1999). During active wakeful states, information coded by neocortical structures flows into the entorhinal cortex and then is encoded by rapidly changing synapses in the hippocampus. During deep sleep, information flows out of the hippocampus, and through repetitive activity, becomes entrained onto slow changing synapses in the neocortex (Marshall & Born, 2007). Thus, sleep may enable the hippocampus to shut off acquisition of new memories and turn on repetitive firing that can allow memories to be encoded permanently in the neocortex (Marshall & Born, 2007; Mehta, 2007).
In another view, sleep may possibly play a more passive role by protecting newly formed memories from retroactive interference (Wisted, 2004). Sleep (along with alcohol, benzodiazepines, and NMDA receptor antagonists) blocks new inputs to the hippocampus without compromising its ability to consolidate previously formed memories. Because new input is prevented, recently formed (and, therefore, incompletely consolidated) memories are protected from the retroactive interference that they would otherwise encounter. Thus, memories have an enhanced opportunity to consolidate during sleep, undisturbed from waking interference.

There also does not appear to be a brief critical time window for sleep to occur after training, as suggested by previous studies (Smith & Rose, 1996, 1997; Smith, Cotway, & Rose, 1998). We found enhancements if sleep occurred during 24 hr posttraining on this task. However, the precise timing may be task specific, so that for contextual fear conditioning, there is a larger time window in which sleep benefits the consolidation process.

As the current novel paradigm controls for circadian variability, passage of time, and stress-induced amnesia, it may prove to be a valuable tool for further examination of the molecular, physiological, and neurobiological substrates of sleep-induced memory consolidation.

References


SLEEP AND FEAR CONDITIONING


Chapter 3

Zolpidem-induced sleep and amnesia are functionally coupled:
dose-effect analysis on Pavlovian fear conditioning

by
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Abstract

With the high prevalence of sleep disorders in our society, there is a growing interest in sleep-aids, with a particular focus on so-called "Z-drugs". The most popular Z-drug is zolpidem, a hypnotic that is very fast-acting and highly potent. While zolpidem does not share the molecular structure of classical benzodiazepines, it binds to the benzodiazepine recognition site of GABA\textsubscript{A} receptors. It shows greater preference, however, for receptors containing $\alpha_1$ subunits, as compared to other sedative-hypnotics, including benzodiazepines. Recent case reports have raised concerns about the side effect profile of zolpidem, highlighting its amnesic properties. In the current study, we performed a dose-effect analysis of the effects of zolpidem on Pavlovian fear conditioning in order to characterize the relationship between the sedative and amnesic effects of the drug. We found that zolpidem induced sleep before amnesia for both context and tone conditioning. These data suggest that the sedative and amnesic effects of zolpidem are functionally coupled and there is no specific amnesia apart from its sedative-hypnotic effects.
Introduction

Insomnia affects about 30% of the general population (Roth, 2007). These symptoms include difficulty initiating sleep, difficulty maintaining sleep, early waking, and nonrestorative or poor quality of sleep (Ancoli-Israel and Roth, 1999). Insomnia is associated with increases in health problems, accidents, costs to society and decreased quality of life (Akerstedt, 2003; Balter and Uhlenhuth, 1992; Foundation, 1991; Katz and McHorney, 2002; McHorney et al, 1994; McHorney et al, 1993; McHorney et al, 1992).

With this high prevalence of insomnia, hypnotics are among the most prescribed medications. In particular, there has been a growing interest in the so called "Z-drugs" to aid sleep-related disturbances, because of these drugs' reduced abuse potential, rapid action, greater selectivity, and preserved sleep architecture compared to older hypnotics. Z-drugs are structurally distinct from benzodiazepines (BZ), but have similar hypnotic effects, and appear to act on the BZ site of the GABA$_A$ receptor. The most popular and potent Z-drug is zolpidem (Ambien®), which is very fast acting (≤15 min) (Atack et al, 2005; Licata and Rowlett, 2008; Morlock et al, 2006; Rowlett et al, 2005; Salva and Costa, 1995). Zolpidem binds to GABA$_A$ receptors, with a high affinity for those containing α$_1$ subunits. Both rodent and human studies have shown zolpidem to increase time spent in stage-2 and slow-wave sleep, and increase latency to rapid-eye movement sleep (Depoortere et al, 1986; Nicholson and Pascoe, 1986). In contrast, zolpidem has reduced anxiolytic properties compared to benzodiazepines (Depoortere et al, 1986).

While the sedative effect of zolpidem is helpful in treating insomnia, there have been reports of troublesome psychiatric side effects, especially when combined with
other drugs. This concern has risen so high that the U.S. FDA has warned that zolpidem is associated with "abnormal thinking and behavior changes...bizarre behavior, agitation, depersonalization...hallucinations...'sleep driving'...having sex...patients usually do not remember these events. Amnesia...may occur unpredictably" Despite this labeling, only 0.5% of patients reported any amnesia (Sanofi-Aventis, 2008).

The memory impairment of BZs, such as triazolam or midazolam, is well-known and often used to clinical advantage, but not well understood (Curran, 1991; Duka et al, 1996; Woods et al, 1992). Research generally suggests that BZs produce anterograde but not retrograde amnesia (Fillmore et al, 2001; Weingartner et al, 1992; Wixted, 2004). While zolpidem is structurally dissimilar to these BZs, it shares a common binding site on the GABA$_A$ receptor, and therefore may produce a similar amnesic effect.

Zolpidem may be decoupled from the anxiolytic effects of BZs, because drugs that have greater affinity for $\alpha_1$ subunit-containing receptors produce more sedation and amnesia than those that favor $\alpha_2$ or $\alpha_3$ subunit-containing receptors, which produce more anxiolysis. Mutant analysis has identified the amnesic and hypnotic effects as dependent on the $\alpha_1$ subunit, while the anti-anxiety effect depends on the $\alpha_2$ subunit (Atack, 2003; Dolder and Nelson, 2008). Therefore, the amnesic and hypnotic effects may be functionally coupled, and thus are more pronounced in ligands that favor $\alpha_1$ subunit-containing receptors, such as zolpidem.

We conducted a dose-response analysis of the effect of zolpidem on Pavlovian fear conditioning. Fear conditioning involves pairing an initially neutral tone with an aversive footshock in a distinct environmental context. After training, rodents will exhibit
fear, measured as freezing, when presented with the training tone, or when returned to the context (Fanselow, 1980; Fanselow and Bolles, 1979). Contextual fear conditioning has garnered considerable interest in recent years because it is hippocampus-dependent and has become a prominent rodent model of declarative memory (Anagnostaras et al, 1999a; Anagnostaras et al, 2000). Pavlovian fear conditioning is acquired rapidly, and can dissociate between hippocampus-dependent and -independent memory. Consistent with human declarative memory, contextual fear gradually becomes independent of the hippocampus, as this memory is consolidated to neocortical structures (Anagnostaras et al, 1999b; Frankland et al, 2004; Maren et al, 1998; Quinn et al, 2008). In contrast, tone (cued) fear is independent of the hippocampus (Anagnostaras et al, 2001), yet both contextual and cued fear memory depend on the amygdala for the lifetime of the animal (Gale et al, 2004).

We administered a range of doses of zolpidem 15 min before training on Pavlovian fear conditioning, and then tested the animals for contextual and cued memory off-drug. We also examined locomotor activity and sleep behavior, predicting that amnesia would appear before animals were sedated. In contrast, the amnesic and hypnotic effects of zolpidem seemed functionally coupled, although hypnosis appeared at slightly lower doses than amnesia. That is, animals had to be in a relatively deep sleep to experience amnesia.

**Materials and Methods**

*Subjects.* A total of 124 hybrid C57BL/6Jx129T2SvEms/J (Crawley et al, 1997; Matynia et al, 2008) male and female mice (approximately equivalent sexes, stock from the Jackson Laboratory, West Sacramento, CA) were balanced across groups. Mice
were in a 14:10 light:dark cycle for the duration of the experiment. All animals were handled five times for one min each prior to the start of the experiment. They had unrestricted access to food and water. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and were approved by the UCSD IACUC.

*Drugs.* Zolpidem tartrate (Sanofi-Aventis, Bridgewater, NJ) was dissolved in 0.9% sodium chloride. Mice were randomly assigned to one of nine zolpidem doses in order to form a base-2 logarithmic dose-response curve: 0 mg/kg (saline controls, n=28), 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg (*n* = 12/zolpidem dose). Injections were given 15 minutes prior to training i.p. (10 ml/kg; free zolpidem weight). These doses were chosen around the prescribed dosage (10 mg, or about 0.15 mg/kg), and 8.0 mg/kg was chosen as the highest dose because it produces deep sleep (Depoortere *et al*, 1986). The LD$_{50}$ for zolpidem is > 600 mg/kg (male rats) so this was not a central concern (Sanofi-Synthelabo, 2001).

*Fear conditioning.* During training, mice were placed into a fear conditioning chamber on-drug (see *Conditioning Context* below). After a 2-min baseline, they were given one tone (2.8-kHz, 30-s, 85-dB)–footshock (2-s, 0.75-mA, AC, scrambled) pairing which co-terminated 2.5 min into training. After an additional 7.5 min, which served as an extended post-shock freezing test, they were returned to their home cages. A week later, mice were tested for contextual memory (off-drug) by placing them back into the original chamber for 5 min. The following day, mice were tested for tone memory (also off-drug) by placing them into an alternate context (see below) for a 2-min baseline period, followed by presentation of three 30-sec tones (same as training), each
separated by 30 sec, without shock. Freezing and gross movement were assessed for
the entire training and testing periods using an automated algorithm (see below).
Experimentally blind observers hand-scored sleep behavior by time-sampling every 8-
sec for the 2 min period prior to the tone-shock period on the training day; sleep was
defined as the mouse having its eyes closed and a lack of all movement except that
required for respiration.

*Conditioning context*. Four mice were tested concurrently, in individual
conditioning chambers housed in a windowless room. Each chamber (32 cm wide, 25
cm high, 25 cm deep) was located within a sound attenuating chamber (63.5 cm wide,
35.5 cm high, 76 cm deep; Med-Associates Inc., Georgia, VT) and equipped with a
speaker in the side wall, a stainless steel grid floor (36 rods, each rod 2-mm diameter, 8-
mm center to center; Med-Associates Inc., Georgia, VT) and stainless steel drop-pan.
During each trial, chambers were scented with 7% isopropyl alcohol to provide a
background odor and background noise (65-dB) was provided by internal fans. Each
sound attenuating chamber was equipped with an overhead LED light source providing
white and near infrared light, and an IEEE 1394 progressive scan video camera with a
visible light filter (VID-CAM-MONO-2A; Med-Associates Inc., Georgia, VT) connected to
a computer and video equipment in an adjacent room. Each chamber was connected to
a solid-state scrambler, providing AC constant current shock, and an audio stimulus
generator, controlled via an interface connected to a Windows computer running Video
Freeze (Med-Associates Inc., Georgia, VT), a novel program designed for the automated
assessment of freezing and motor activity. In results that will be published more fully
elsewhere, computer and human scored data had a correlation of 0.971 and a fit of
computer = −0.007 + 0.974 x human (for additional detail see for e.g., Anagnostaras et al, 2000; Shuman et al, 2009; Wood and Anagnostaras, 2008).

Alternate context. For testing cued (tone) fear, the conditioning context was modified along several dimensions. White acrylic sheets were placed over the grid floor to provide a different sensory experience and a black plastic, triangular tent translucent only to near infrared light was placed inside each box, with each side of the triangle measuring 23cm. Only near infrared light was used creating a completely dark environment visible only to the video camera. Between tests, the chambers were cleaned and scented with a 5% white vinegar solution.

Statistics. Data were entered into an analysis of variance (ANOVA). Generally, following a significant omnibus univariate ANOVA for each dependent measure, multiple post-hoc comparisons were made using Fisher’s protected least significant difference (PLSD), comparing each dose with the saline control group (SPSS 16, SAS Software, Cary, NH; Prism 5.0, Graphpad Software, La Jolla, CA). The level of significance was set at \( p \leq 0.05 \).

Results

Baseline Sleep. There was a dose-dependent effect of zolpidem on sleep during the 2-min of baseline training (Fig 1A). Although the definition of sleep partially overlaps with freezing, the correlation between sleep and freezing during the training session suggests these measures are related, yet different \( (r^2 = 0.47, p < 0.0001) \). There were significant group differences in percent of time sleeping \( [F(8, 115) = 35.9, p \leq 0.0001] \). Doses of zolpidem between 0.5-8 mg/kg of induced more sleep compared to saline
controls (Fisher’s PLSD, \( p \) values \( \leq 0.05 \)). No other groups were significantly different than controls (\( p \) values \( > 0.05 \)).

**Baseline Activity.** Zolpidem produced a dose-dependent decrease in baseline locomotor activity during the first 2-min of training (Fig 1B). A one-way ANOVA revealed group differences \( [F(8, 115) = 19.8, p \leq 0.0001] \). Subjects administered 0.5-8 mg/kg of zolpidem pre-training displayed significantly decreased activity compared to saline controls (\( p \) values \( \leq 0.01 \)). Indeed, doses of 1 mg/kg and above virtually abolished locomotor activity. No other groups were significantly different than the control group (\( p \) values \( > 0.05 \)).

**Shock Reactivity.** There were group differences in shock reactivity, which is measured by locomotor activity during the 2-s footshock \( [F(8, 115) = 23.7, p \leq 0.0001] \). Surprisingly, 0.01 and 0.05 mg/kg substantially increased motor reactivity to the shock (\( p \) values \( \leq 0.01 \)), without affecting baseline locomotor activity or sleep (see above); in contrast 2-8 mg/kg of zolpidem abolished shock reactivity when compared to saline controls (\( p \) values \( \leq 0.0001 \)). This suggests that doses of 2 mg/kg and above produce a profoundly deep sleep, to the point that mice could not be aroused with footshock. Other groups were not significantly different than the control group (\( p \) values \( > 0.05 \)).

**Training.** Figure 2A depicts the first 5 min of training, consisting of a 2-min baseline period, followed by a 30-s tone co-terminating with a 2-s footshock at 2.5 min. There was a significant main effect of group \( [F(8, 115) = 30.6, p \leq 0.0001] \) and dose-by-minute interaction \( [F(32, 115) = 19.8, p \leq 0.0001] \). Post hoc comparisons revealed a
dose-dependent increase in freezing for subjects that received 0.5-8 mg/kg of zolpidem ($p$ values $\leq 0.0001$). No other groups were significantly different than controls ($p$ values $> 0.05$).

*Post-shock freezing.* In minutes 6 through 10 of training, freezing was measured with no further presentation of tone or shock, as an index of post-shock freezing or immediate memory (Fig 2B; Anagnostaras *et al*, 2003). There were significant group differences [$F(8, 115) = 13.9, p \leq 0.0001$]. Post hoc comparisons revealed a dose-dependent increase in freezing for subjects that received 0.5-8 mg/kg of zolpidem ($p$ values $\leq 0.0001$). No other groups were significantly different than the control group ($p$ values $> 0.05$). Overall, these data suggest that effects on freezing during training (when mice were on-drug) are directly related to the drug's effect on locomotor activity and sleep. For example, 0.5 mg/kg partially attenuated baseline locomotor activity (Fig 1B), partially induced sleep (1A), and produced a small increase in observed freezing. Doses of 1 mg/kg and above profoundly decreased activity, increased sleep, and freezing.

*Contextual Memory Test.* Mice were returned to the original training chambers seven days after training, for an off-drug memory test. Freezing was measured for 5 min without any presentation of the tone or shock (Fig 3A). Zolpidem-trained animals had a dose-dependent impairment [$F(8, 115) = 9.3, p \leq 0.0001$], such that doses of 1-8 mg/kg during training impaired contextual fear memory compared to saline controls ($p$ values $\leq 0.0001$). No other groups were significantly different than controls ($p$ values $> 0.05$). Therefore, doses that produced a lot of sleep during training (Fig 1B), also produced deficits in contextual fear memory. One dose (0.5 mg/kg), however, induced some sleep
but failed to produce a deficit in contextual memory. This indicates that the amnesic effect of the drug requires a higher dose than the hypnotic effect.

*Tone Memory Test.* Twenty-four hours after the contextual memory test, mice were placed into the alternate context. Freezing was measured for 5 min, consisting of a 2-min baseline period followed by three 30-s presentations of the training tone (Fig 3B). Tone memory was measured as freezing during the baseline subtracted from the average freezing to the 3 tones. There was a dose-dependent impairment \( [F(8, 115) = 2.0, p \leq 0.05] \), as high doses (2 and 8 mg/kg of zolpidem during training) impaired tone memory compared to saline controls \( (p \text{ values} \leq 0.0001) \). No other groups were significantly different than the control group \( (p \text{ values} > 0.05) \).

*Median Effective Dose Calculations.* Based on the preceding findings, it appeared that zolpidem induced sleep (0.5-8 mg/kg) before having an effect on either context (1-8 mg/kg) or tone memory (2 and 8 mg/kg). In order to further examine this relationship, the median effective dose was calculated for the sleep inducing effects, as well as the contextual and cued fear memory deficits. Median effective dose was calculated according to previous methods (Anagnostaras et al, 1999b; Nies, 1993): 1) sleep induction was defined as one-half increase (\%sleep), relative to the mean of saline controls; amnesia was defined as a one-half decrease (\% freezing) relative to the mean of saline controls, for context and tone separately. As such, animals in each group that had more than (sleep) or less than (freezing) half of the average control responses were assigned a value of 1, and all other animals were assigned a zero; 2) probability-dose curves were then generated (Fig 4A-C) and logarithmic curve fits (excluding saline
controls) were made; and 3) the $ED_{50}$ for each effect was computed from the best curve fit (Prism 5.0, Graphpad Software, La Jolla, CA).

Figure 4A-C depicts the probability of observing sleep (one-half increase in baseline sleep) or amnesia for tone or contextual memory (one-half decrements in context or tone memory). The lowest dose was required for sleep induction, with about 0.40 mg/kg zolpidem inducing sleep in half of the subjects (Fig 4A). For context, about 0.71 mg/kg was required to produce amnesia (Fig 4B). For tone, a lower, but very similar, dose of 0.54 mg/kg was required (4C). These results agree with the previous calculations, where significant deficits were in the range of 1-8 mg/kg and 2-8mg/kg for contextual and tone tests, respectively, and the affected range for baseline sleep was 0.5-8 mg/kg. The results from the analysis of variance and median effective dose calculations converge on the conclusion that zolpidem induces sleep before amnesia for both context and tone conditioning. Nonetheless, the $ED_{50}$s fall within similar dose ranges, suggesting the effects are functionally coupled.

**Discussion**

Consistent with evidence that zolpidem can produce amnesia, high doses of zolpidem during training impaired memory of context and tone fear when mice were tested off-drug one week later. These doses, however, also induced profound amounts of sleep, with accompanying reductions of locomotor activity. While impairments for context and tone memory did not occur until doses reached 1 and 2 mg/kg, increased sleep at training began at 0.5 mg/kg. Despite the animals being more sedated at 0.5 mg/kg, this dose did not cause deficits in context and tone memory, nor did any of the lower doses. Indeed, at 2 mg/kg and above, sleep was so deep that mice exhibited no
reaction whatsoever to footshock (Fig 1C). Overall, this data suggests that sleep and amnesia are functionally coupled, with sleep occurring just before anterograde amnesia sets in.

Prior human studies have found that it takes at least 15-20mg of zolpidem to reliably impair a variety of memory tasks, including verbal recall and recognition, digit substitution, psychomotor testing, etc (Mattila et al, 1998; Mintzer and Griffiths, 1999a, b; Roehrs et al, 1994; Wesensten et al, 1995). However, these doses are higher than what is typically prescribed for sleep induction (10 mg), as at 15-20 mg there is an increase in sedation, increased subjective reports of sleepiness (Mintzer et al, 1999b) a subjective feeling of being drugged (Mattila et al, 1998), decreased latency to sleep (Roehrs et al, 1994), decreased latency to stage 2 sleep (Wesensten et al, 1995), and an increase in the amount of slow-wave sleep (Wesensten et al, 1995). Despite finding amnesic and hypnotic effects occurring at the same doses, few studies have tied the two together to suggest that the memory impairments are a result of not being fully awake to process information. It is unreasonable to expect sleeping subjects to learn as well as awake subjects. Therefore, we believe that an assessment of sleep status is an essential component of studies examining amnesia and drugs acting at GABA\textsubscript{A} receptors.

Consistent with previous rodent studies, hypnotic effects, such as decreases in locomotor activity, result even from low doses of zolpidem, (≤ 0.5 mg/kg). However, memory deficits on active avoidance and punished drinking do not appear until doses of 1 mg/kg or higher (Depoortere et al, 1986; Sanger et al, 1986). While the exact translation between rodent and human dosing is unclear (Shuman et al, 2009; Wood and Anagnostaras, 2009; Wood et al, 2007), this dose appears to be far higher than that
prescribed for humans, which is about 0.15 mg/kg. The current study, taken together with the body of literature to date, strongly suggests that zolpidem does not cause anterograde amnesia separate from its hypnotic effects, such as sleepiness. In fact, a 0.5 mg/kg dose increased sleep, yet it did not affect either context or tone memory, as further evidence for the lack of amnesic effect of zolpidem apart from sleep.

We speculate that rapid onset hypnotics, such as zolpidem, may induce a state in which subjects look awake but are actually in a hypnagogic sleep-like state (Dolder et al, 2008). While there is little published evidence on the EEG patterns of patients treated with rapid onset hypnotics just prior to falling asleep, there are reports of patients appearing awake (such as those undergoing surgical sedation) (Persson et al, 1988). However, it seems unlikely that time period would be characterized by alert-type b EEG activity. This hypnagogic state may be characterized by amnesia (Dolder et al, 2008; Persson et al, 1988).

Consistent with this idea, studies using targeted genetic mutations have found that the hypnotic and amnesic effects of drugs that act at the BZ site are through receptors that contain the α1 subunit. Thus, given current knowledge of GABA_A pharmacology, it is not presently possible to decouple hypnosis and amnesia. This is in contrast with the anxiolytic effects which appear to be mediated through receptors with α2 subunits, and are already functionally decoupled in zolpidem (Atack, 2003; Depoortere et al, 1986).

The case reports of zolpidem-inducing amnesia may be due to an interaction of mixing zolpidem with other drugs, especially alcohol or other sedative-hypnotics (Dolder
et al, 2008). These drugs can intensify the effect of zolpidem, resulting in amnesia because of a higher effective dose, or action at multiple sites. Approximately 40% of adults with insomnia also have a diagnosable psychiatric disorder—most notably depression and anxiety (Roth, 2007). It is likely, then, that a person with comorbid psychiatric disorder may also be taking other prescription drugs, in addition to zolpidem. Finally, since reports of amnesia and various unusual behaviors with zolpidem are fairly rare, they could be due to atypical individual response patterns. For example, zolpidem could induce sleep disorders such as somnambulism, which is accompanied by amnesia, in certain sensitive patients (Dolder et al, 2008). It does not, however, appear that zolpidem produces significant amnesia in most cases, beyond that expected from sedation. The present study suggests that the explosion of case reports of amnesia from this widely prescribed drug is due to polydrug use or rare, atypical reactions.
Figures

Fig 1.

Figure 1. A. Baseline Sleep. We measured sleep during the 2 min baseline prior to any shock. Sleep was hand scored by naive observers. A dose-dependent effect of zolpidem was apparent, with 0.5-8 mg/kg inducing more sleep than compared to saline controls. B. Baseline Activity. Computer scored movement is depicted (mean arbitrary units ± SEM) during the 2 min baseline prior to any shock. Doses between 0.5-8 mg/kg decreased activity compared to saline controls. C. Shock Reactivity. The response to shock was measured by the locomotor activity during the 2-s footshock. Low doses of zolpidem (0.01 and 0.05 mg/kg) increased shock reactivity, while high doses (2-8 mg/kg) abolished the response to the shock when compared with saline controls.
Figure 2. Training. Mice were given fear conditioning 15 min after the administration of zolpidem or saline. A 2 min baseline was followed by a tone-shock pairing (min 2-2.5). Freezing (mean % time±SEM) is depicted for each minute of the training session on-drug. There was a dose-dependent effect, with 0.05-8 mg/kg increasing freezing more than saline controls. B. Post-shock. Freezing for the last 5 min of training was averaged for each group. There was a dose-dependent increase in freezing, with 0.5-8 mg/kg freezing at higher levels than saline controls.
Figure 3. Context Test. A week after training, mice were brought back to the original conditioning chambers off-drug, where neither shock nor tone was present. Freezing (mean %time±SEM) for the 5 min test was averaged for each group. There was a dose-dependent decrease in context memory, with 1-8 mg/kg freezing less than saline controls. B. Tone Test. A day after context test, mice were brought to a novel context for a 5 min tone test off-drug. After a 2 min baseline period, the training tone was presented 3 times across 3 min. Cued fear is depicted as freezing (mean %time±SEM) during the baseline subtracted from the average of freezing to the 3 tones. High doses (2-8 mg/kg) impaired tone memory.
Figure 4. Median Effective Dose Calculations. For each response, probabilities of sleep induction (one-half increase in sleep) or context or tone amnesia (one-half reduction in freezing), relative to the mean of saline controls was calculated for each dose; after a logarithmic curve fit was made, median doses for each effect was calculated. A. Baseline Sleep. About 0.4 mg/kg was sufficient to induce sleep during training in one half of the subjects. B. Context Test. About 0.71 mg/kg was required to induce amnesia for contextual memory. C. Tone test. About 0.54 mg/kg was required to induce amnesia for tone memory. Overall, these data suggest that zolpidem-induced sleep and amnesia appeared at similar doses, with sleep appearing first, suggesting that sleep is functionally coupled to amnesia.
Disclosure/ Conflicts of Interests

The author(s) declare that no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.
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References


Supplementary Materials

Fig 1.

Figure 1. Schematic of design. Mice were trained on Pavlovian fear conditioning prior to their awake or sleep phase. Immediately after training, they were administered 8mg/kg of zolpidem or saline and put back in their home cage. Twelve hours after training, mice were tested for context and cued memory.
Figure 3. Context and cued memory tests. (A) Contextual fear. Freezing (mean percent time plus standard error of the mean) for the 5-min test is depicted. Mice with zolpidem induced deep sleep during the awake phase had stronger contextual memory than control mice that had an awake phase. Pharmacologically inducing sleep during the sleep phase did not alter contextual memory. (B) Cued fear. One hour after the context test, mice were brought to a novel context for a 5-min cued fear test. After a 2-min baseline period, the training tone was presented three times across 3 min.
Cued fear is depicted as freezing (mean percent time plus standard error of the mean) during the average of freezing to the three tones. There were no group differences on the cued test.
Chapter 3, in part, has been submitted for publication of the material as it may appear in Zolpidem-induced sleep and amnesia are functionally coupled: dose-effect analysis on Pavlovian fear conditioning. *Psychopharmacology.* Cai, D.J., Shuman, T., Sage, J.R., & Anagnostaras, S.G. (in revision). The dissertation author was the primary investigator and author of this material.
Chapter 4

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Research report

Comparing the benefits of caffeine, naps and placebo on verbal, motor and perceptual memory

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A B S T R A C T

Caffeine, the world’s most common psychoactive substance, is used by approximately 90% of North Americans everyday. Little is known, however, about its benefits for memory. Napping has been shown to increase alertness and promote learning on some memory tasks. We directly compared caffeine (200 mg) with napping (50–90 min) and placebo on three distinct memory processes: declarative verbal memory, procedural motor skills, and perceptual learning. In the verbal task, recall and recognition for unassociated words were tested after a 7-h retention period (with between-session nap or drug intervention). Ascending different word lists was administered post-intervention and memory was tested after a 20-min retention period. The non-declarative tasks (tongue tapping task (FTT) and texture discrimination task (TDT)) were trained before the intervention and then retested afterwards. Naps enhanced recall of words after a 7 h and 20 min retention interval relative to both caffeine and placebo. Caffeine significantly impaired motor learning compared to placebo and naps. Napping produced robust perceptual learning compared with placebo; however, naps and caffeine were not significantly different. These findings provide evidence of the limited benefits of caffeine for memory improvement compared with napping. We hypothesize that impairment from caffeine may be restricted to tasks that contain explicit information; whereas strictly implicit learning is less compromised.

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1. Introduction

Caffeine, the world’s most widely consumed stimulant [1], is an active ingredient in coffee, tea, chocolate, sodas, and energy drinks (the fastest growing sector of the American beverage industry) [2]. Modern times have led to an increase in daily, often multiple doses of caffeine, a rise in the coffee business, and the addition of caffeine to common beverages such as soda, bottled water, and even chewing gum. Based on the available product usage and food consumption data, Barone and Roberts [3] estimated the mean daily intake was 4 mg/kg body weight (approximately 280 mg for a 155 pound person; 16 ounces of Starbucks coffee contains 372 mg). For the 90th percentile of caffeine users, intake approximated 5–7 mg/kg body weight (approximately 300–500 mg).

This increasingly common use of caffeine in our society coincides with an increasingly common trend of individuals obtaining insufficient sleep on a regular basis. While it is difficult to ascertain the exact number of individuals who use caffeine as a substitute for sleep in society, the 2005–2007 National Sleep Foundation’s annual Sleep in America polls strongly suggest that Americans regularly consume caffeine as a substitute for sleep and/or as a result of insufficient sleep (4–6). These polls report consistent associations between low quantity or quality of sleep, decreased daytime functioning, and increased daytime caffeine consumption.

A number of studies have examined the benefits of daytime caffeine consumption in non-experimentally sleep-deprived individuals [7–19]. The performance tasks used in these studies measure reaction time and motor speed, speed of information processing, vigilance and attention, immediate and delayed verbal memory, as well as mood and alertness (for review see [10,18]. Generally, caffeine enhances mood and alertness [8,9], speed of information processing [14,19], reaction time and motor speed [8,9,14,19]. One study found 200 and 300 mg of caffeine benefited visual vigilance, choice reaction time, repeated acquisition, and self-reported fatigue and sleepiness, but did not improve marksmanship, a task that requires fine motor coordination and steadiness [16,17]. Dunlop et al. measured the effects of placebo, 200 and 400 mg of caffeine on human
While these studies show caffeine can enhance wakfulness and performance on attention and concentration tasks, little agreement may be found in the literature on caffeine and memory [7,19,21]. In their review, Nehlig et al. [19] write "In man, memory per se is not improved but response tends to be quicker and less errorful with caffeine." An alternative explanation for the negative findings is that only a limited number of memory processes have been examined. A thorough examination of the effects of caffeine across a wide range of memory processes has not been completed. Thus, it is still an open question whether caffeine improves learning and memory [11,12], either more generally or in specific memory domains.

Naps, in contrast to caffeine, have been shown to enhance not only alertness and attention, but also some forms of memory consolidation. In particular, naps (daytime sleep between 5 and 90 min) appear to improve performance on non-medio-temporal lobe dependent, procedural skills [22–25]. Mednick et al. reported that a mid-day nap can also reverse perceptual deterioration that builds with repeated within-day testing [22]. They further showed that naps with SWS and REM produced improvements in performance equivalent to that of a full night of sleep, whereas naps with only SWS restored deteriorated performance to baseline levels [23]. Walker and coworkers have demonstrated that naps improve procedural motor skill learning to the same degree as a full night of sleep, and that improvement on this task was correlated with Stage 2 and 2 sleep spindle activity [25,26]. Tucker compared naps with non-REM sleep to a no-sleep condition on a procedural motor task and a declarative, verbal-paired-associates task. They found that the non-REM naps produced improved performance in the declarative, but not the procedural task [27]. This is evidence that non-REM is less likely to produce declarative memory improvements as nocturnal non-REM sleep [28].

Prior studies of performance during nightshift work have directly compared caffeine and napping in one of a variety of tasks [29,30]. For example, recently, Sagaras et al. compared the effects of a single 200 mg dose of caffeine to a 30 min nap and placebo on nocturnal driving in young and middle-aged participants. They found that both interventions significantly improved performance in both age groups, although napping was even more effective in younger compared to older participants. There are no studies, however, directly comparing the effects of caffeine and naps during the day in normally rested individuals, and few that have compared caffeine and sleep at any time for cognitive processes beyond attention, vigilance, or driving. Here, we compared the effects of caffeine, a daytime nap, or placebo on three distinct memory processes: declarative verbal memory, procedural motor skills, and perceptual learning. For verbal memory, we tested recall and recognition in two different phases: 7 h retention with a between-session intervention (caffeine, placebo or nap), and 20 min retention for a different list of words post-intervention. The non-declarative tasks (finger tapping task [FTT] and texture discrimination task [TDD]) were trained before the intervention and then retested afterwards.

2. Methods

2.1 Subjects

61 adults between ages 18–59 with no personal history of neurological, psychological or other chronic illness (non-smoking) gave informed consent to participate in the experiment, which was approved by the institutional review boards of the University of California at San Diego. Subjects were low to moderate caffeine drinkers (no more than two cups of coffee per day). Those restricted to nighttime sleep can have a deleterious effect on performance [31]; we required that subjects maintain a sleep schedule for one week prior to the study. These seven nights prior to the study, subjects were instructed to go to bed no later than midnight and to get up no later than 8 a.m. They were asked to spend at least 8 h in bed each night. Subjects filled out sleep diaries and were asked to subjectively and objectively measure of sleep-wake activity. Subjects were restricted from consuming caffeine and alcohol 24 h prior to and during the experimental day.

An uneven number of subjects were run in all three tasks due to technical error, subjects misunderstanding the task which led to unusable data, and adding the verbal task midway through the study for the Verbal task, 11 placebos, 12 napppers and 12 caffeine subjects were run. For the Motor task, 10 placebos, 11 napppers and 10 caffeine subjects were run. For the Perceptual task, 19 placebos, 18 napppers and 18 caffeine subjects were run.

2.2 Study Procedures

Fig. 1 shows study timeline (an example task order scenario). Task order was counterbalanced across subjects. Subjects were in the lab under supervision during the entire experimental day. Subjects' knowledge of testing procedure was limited to being told that they would be tested in the morning and afternoon on all three tasks. At 09:30, subjects were administered the initial verbal task and were trained on the finger tapping task and texture discrimination task (Session One). Lunch was served at noon. At 11:00, subjects were randomly assigned to a nap or a drug group. Subjects either took a polysomnographically (PSG) recorded nap (30- min of sleep maximum or up to 1 h in bed) or listened to a book on tape with PSG monitoring. A summary of nap PSG can be found in Table 1. At 15:00, subjects in the drug groups were given an unmarked pill (200 mg caffeine or placebo). Sixty minutes later (Session Two), subjects were tested on all three tasks, as described below.

In addition, subjective sleepiness was measured before and after each test session with the Karolinska Sleepiness Scale (KSS). The KSS assesses subjects' momentary state of alertness/sleepiness on a 1–9 scale ("extremely sleepful"). Before the first test session subjects also completed the Epworth Sleepiness Scale. The Epworth assesses trait daytime sleepiness with eight questions, each scored with a degree of severity ranging from 0 to 3. A score less than 10 is considered normal. Table 2 shows the demographic information, Epworth

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Polyomorphometric of naps (mean and standard deviation)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>DST</td>
</tr>
<tr>
<td>09:30 ± 0.23</td>
<td>0.38 ± 0.4</td>
</tr>
</tbody>
</table>
score, and articulatory data from the week prior to experimental day, including Total Sleep Time (TST), BEDtime, and WakeTime.

2.3. Verbal task

We examined recall and recognition memory in two different phases of verbal memory: 7-h retention with a between-session intervention (caffeine, placebo or nap), and 20-mm retention for a different list of words post-intervention. During Session 1, subjects were trained and tested on Word List 1. During training, the experimenter read aloud 24 unrelated words in three consecutive trials. Immediately after each trial, subjects were asked to recall the words. After a period of 20 min (during which non-verbal tasks were completed), subjects were given tests of free recall and recognition for Word List 1. No feedback on performance was given. In the recognition test, subjects were read aloud a list of 48 words (half the words were Word List 1 and half were foils) and determined which were on Word List 1.

At the start of the second test session, tests of recall and recognition were given for Word List 1 in order to test for 7-h retention. Afterwards, the entire verbal memory task was repeated with Word List 2 to test for 20-mm retention on recall and recognition memory. For each test session, free recall was measured as the number of words correctly recalled unprompted. Recognition memory performance was measured with ‘d’ (index of discriminability between target and base words). We used two of the word lists here that were previously developed for other studies of verbal learning in our lab [34]. Words were chosen from these norms for recallability by Christian et al. [33], and each list was matched for recallability, word length, concreteness, and imagery. List order was counterbalanced across subjects.

2.4. Motor task

The finger tapping task was identical to that from Walker et al. [34]. The task required subjects to repeatedly complete, with their left (non-dominant) hand, the sequence 4-1-3-2-4 on a keypad. Each block consisted of 30 s of key presses followed by 30 s of rest. The training session consisted of 12 blocks and the test session consisted of 5 blocks. The numeric sequence (4-1-3-2-4) was displayed at the top of the screen at all times to exclude any working memory component to the task. Each key press produced a white dot below, forming a row from left to right over the course of each key press sequence. Performance was measured as the number of correct sequences completed (score), and number of errors made (accuracy).

2.5. Perceptual task

Participants performed a tone discrimination task similar to that developed by Karan and Tajj [57] and identical to that utilized in our previous studies (22, 23, 30). Participants were asked to discriminate two targets per trial: a central letter (‘T’ or ‘L’), and a peripheral line array (vertical or horizontal orientation) on the lower left quadrant at 2.5–3.5 mm eccentricity from the center of the screen. The peripheral array consisted of three diagonal bars that were either positioned in a horizontal array or a vertical array against a background of horizontally oriented bars, which created a texture-difference between the target and background.

An experimental trial consisted of the following sequence: central fixation cross, target screen for 32 ms, blank screen for a duration between 0 and 600 ms (the inter-stimulus-interval, or ISI), mask for 30 ms followed by the response time interval before the next trial. Subjects reported both the letter at central fixation (‘T’ or ‘L’) and the orientation of the peripheral, three-element array (horizontal or vertical) by making two key presses. The central task controlled for eye movements.

Each block consisted of 50 trials, each with the same ISI, and lasting approximately 2 min. A threshold was determined from the performance across 20 blocks, with a progressively shorter ISI, starting with 600 ms and ending with 3 ms. The specific sequence of ISIs across an entire session was 600, 500, 400, 300, 250, 200, 175, 150, 125, 100, 80, 60, 40, 30, 25. A psychometric function of percent correct for each block was fit with a Weibull function to determine the ISI at which performance yielded 80% accuracy.

Participants were instructed to hold each block and were instructed to take as many breaks as they needed between blocks. Once a block began, a new trial initiated every 2 s, regardless of whether or not the subject made a response. Training, which occurred at the beginning of the 9 a.m. test session, consisted of 15 trials of an easy version of the task (10 of 1000–1500 ms), and 50 trials of the easiest block of the actual task (125 of 0.5 ms). This training ensured that participants understood the task and were discriminating the peripheral target between 90% and 100% correct on the easiest version of the task.

3. Analysis

3.1. Verbal task

Our main outcome of interest involved the recall and recognition memory scores for the 7-h retention interval, since that interval included the different interventions. To examine that, we utilized a one-way analysis of variance (ANOVA) using three levels of the variable Group (caffeine, placebo, or nap), separately for recall and recognition. One concern with this approach, though, would be whether the three groups showed equal performance at baseline (i.e., 20 min memory for Word List 1). Thus, we first evaluated that question with a similar one-way ANOVA. If that analysis showed a significant main effect of group, we planned to control for base-line performance by examining the Session X Group interaction in a Repeated-Measures ANOVA. However, since neither 20 min recall nor recognition showed baseline differences (see Section 4, below), we utilized the one-way ANOVAs for the 7-h retention interval to maximize power for our main effect of interest. Finally, to examine the impact of the intervention on the ability to encode new words, we conducted the same analysis for recall and recognition of Word List 1 at the 20 min retention interval. Significant ANOVAs were followed-up by examining differences between groups at the specific time point with independent samples t-tests.

3.2. Motor task

Prior to conducting the response time (RT) analyses described below, errors and a small number of extreme outlier trials (RTs of greater than 3000 ms) were excluded [34]. We examined group differences across Session One and Two with a Repeated-Measures ANOVA, with Group as the between-subjects variable, and Session (mean performance from last two blocks of the training vs. two blocks of test) as the within-subjects variable. This ANOVA was conducted for both score and accuracy.

3.3. Perceptual task

We examined group differences across Session One and Two with a Repeated-Measures ANOVA with Group as the between subjects variable, and session thresholds as within-subjects variables [23].

3.4. Subjective sleepiness

Sleepiness was examined with a mixed model Repeated-Measured ANOVA with Group as the between variable and the four administrations as the within-factors. Also, we specifically examined the KSS rating from immediately after the treatment in a one-way ANOVA to examine acute treatment effects of subjective sleepiness.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>% Female</th>
<th>Education (years)</th>
<th>Sleeptime</th>
<th>TST (minutes)</th>
<th>BEDtime</th>
<th>WakeTime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>23.1 (0.78)</td>
<td>99 (12)</td>
<td>15.1 (0.46)</td>
<td>6.37 (0.42)</td>
<td>455 (75)</td>
<td>23.36</td>
<td>7.26</td>
</tr>
</tbody>
</table>
4. Results

4.1. Verbal task

No significant differences were found between groups in Recall of Word List 1 at 20 min (Recall means and standard deviations = 13.70(3.0), 15.25(3.3), 12.25(3.5) for placebo, nap, caffeine, respectively; F = 2.36, p = 0.11, partial $\eta^2 = 0.12$) or recognition of Word List 1 at 20 min (recognition means and standard deviations = 4.5(0.99), 4.0(0.60), 4.5(0.73), for placebo, nap, caffeine, respectively; F = 0.73, p = 0.49, partial $\eta^2 = 0.04$). Recall memory for Word List 1 after 7 h retention interval showed significant group differences (F = 5.41, p = 0.009, partial $\eta^2 = 0.25$, Fig. 2a). Post hoc tests showed: (a) the nap group performed significantly better than the caffeine group (p = 0.06), (b) nap performed marginally better than placebo (p = 0.03), and (c) there were non-significant differences between caffeine and placebo (p = 0.22). Recognition memory for words after a 7 h retention interval also showed significant group differences for $d'$ (F = 4.51, p = 0.019, partial $\eta^2 = 0.22$, Fig. 2b). Post hoc tests showed: (a) nap performed significantly better than caffeine (p = 0.008); (b) nap better than placebo (p = 0.03); and (c) no difference between caffeine and placebo (p = 0.50).

Recall after a 20 min retention interval showed significant group differences (F = 4.97, p < 0.01, partial $\eta^2 = 0.24$, Fig. 2c). Post hoc tests showed: (a) nap performed significantly better than caffeine (p = 0.004); (b) no difference between nap and placebo (p = 0.21), and (c) caffeine performed marginally worse than placebo (p = 0.08). For recognition memory after a 20 min retention interval, no Group differences were found for $d'$ (F = 0.57, p = 0.57, partial $\eta^2 = 0.03$, Fig. 2d). Data from the Verbal task is shown in Fig. 2.

4.2. Motor task

A Repeated-Measures ANOVA on accuracy showed no significant interaction between group and accuracy (F = 1.87, p = 0.16, partial $\eta^2 = 0.07$). Accuracy was consistently high for all groups. Mean accuracy for the last two blocks of training was 0.97, 0.97, and 0.98 for the placebo, nap and caffeine groups, respectively. For the two blocks of the test session, these values were 0.98, 0.98, and 0.98.

A Repeated-Measures ANOVA on score was statistically significant (F = 6.14, p = 0.004, partial $\eta^2 = 0.21$). Post hoc one-sample t-tests on the differences scores (last two blocks of train session minus first two blocks of test session) indicated the caffeine group showed significantly impaired learning (i.e., smaller increase in the number of sequences completed at Session Two) compared with placebo (p = 0.003), and no difference was found

![Fig. 2](image-url)

Fig. 2. Declarative verbal memory task. Verbal memory performance in placebo (black bar), nap (striped bar), and caffeine groups (gray bar) (means and standard errors), with p-values of significant group differences. Seven hours retention of Morning Words in Recall (a) and recognition in $d'$ (b), 20 min retention of Evening Words in Recall (c) and recognition in $d'$ (d).
in between nap and placebo (p = 0.38). Indeed the caffeine group did not show improvement across sessions (p = 0.43), whereas nappers (p = 0.008) and placebo (p = 0.000) groups showed significantly higher scores. Difference scores are shown in Fig. 3.

4.3. Perceptual task

Performance improvement across the three groups was examined with a Repeated-Measures ANOVA. There was a marginally significant difference across the three groups (F = 2.44, p = 0.09, et$^{2} = 0.09$). Post hoc one-sample t-tests on the difference scores indicate that nappers showed the typical improvement on the TDT compared with placebo (p = 0.02). However, the caffeine group fell in between naps and placebo and was not significantly different from either naps (p = 0.29) or placebo (p = 0.26). Difference scores are shown in Fig. 4.

Fig. 3. Motor skill learning. Differences scores on finger tapping task represent increase in number of correct sequences completed in Session Two compared with Session One in placebo (black bar), nap (striped bar) and caffeine (grey bar) groups.

Fig. 4. Perceptual learning task. Threshold difference score for texture discrimination task represents change in threshold from Session One to Session Two in placebo (black bar), nap (striped bar) and caffeine (grey bar) groups.

4.4. Subjective sleepiness

There was a marginally significant group effect on subjective sleepiness ratings across all four administrations (F = 2.77, p = 0.07, et$^{2} = 0.09$). Compared to naps and placebo, caffeine subjects reported being more alert immediately prior to the testing session following the intervention. Sleepiness rating show significant group differences (F = 3.95, p = 0.03, et$^{2} = 0.20$, Fig. 5) during this third administration.

5. Discussion

In this study, we find that a moderate dose of caffeine impaired motor sequence learning and declarative verbal memory compared to placebo and daytime sleep. These decreases were found despite the fact that caffeine increased subjective alertness, suggesting that the caffeine dose was sufficiently high to have some psychoactive effect. An afternoon nap, on the other hand, improved free recall memory relative to the caffeine group after both a 20 min and a 7th retention interval and produced greater learning on a motor sequence task than caffeine. Although napping produced improvements in the perceptual and motor tasks similar to that previously reported, we also found large amounts of learning in the placebo condition. In the perceptual task, the placebo group showed significantly better performance than previous studies have reported in the non-nap control groups [23]. Specifically non-nap controls typically show performance deterioration with repeated testing, whereas the placebo controls in the present study showed no deterioration. Furthermore, the level of improvements on the motor task in the placebo group is larger than control group performance in prior studies for both nocturnal sleep [34] and naps [25]. We hypothesize, at least for the motor and perceptual tasks, that the placebo condition produced a true “placebo effect” on these memory tasks.

5.1. Napping and memory

Data from the verbal memory task suggest a sleep-dependent consolidation process occurs during the nap that allows for
better recall and a finer discrimination between targets and distracters than can be achieved when sleep does not occur between study and test periods or by caffeine. It should be noted that the present findings suggest a possible role for sleep during naps in declarative memory consolidation of unassociated, rather than associated, words. Recent research has shown that associative and non-associative declarative memory may rely on separate brain regions [37–39]. Specifically, these studies assign item memory formation (non-associative) to the parahippocampal gyrus (particularly rhinal cortices: anterior parahippocampal gyrus and parahippocampal cortex) and associative memory formation to the hippocampus. The majority of prior studies examining parahippocampal-related, sleep-dependent memory have investigated memory for associated word-pairs [40–42]. Instead, the present study examined item memory consolidation, which relies on parahippocampal and rhinal cortices. These findings expand the growing literature on the relationship between memory and sleep to suggest that sleep may be necessary for declarative memory consolidation not limited to processes subserved by the hippocampus itself. In addition to enhancing memory consolidation for previsously studied words, naps improved the ability to learn a new list of words post-intervention when compared with caffeine.

5.2. Caffeine and memory

In this study, caffeine decreased subjective sleepiness. This enhanced alertness, however, did not seem to transfer to motor learning and verbal memory. Although 200 mg of caffeine is considered a moderate dose, other studies have also found similar doses impaired motor skill. In a complex test of hand-eye coordination in which subjects had to insert a stylus successively into three holes, 60 or 120 mg of caffeine decreased, while 180 or 240 mg of caffeine increased the time-to-task-completion [43]. In another study, reaction time increased by 7 mg/kg of caffeine per kilogram of body weight [44]. Other studies have similarly shown that caffeine is unable to reverse the effects of sleep-deprivation on areas of higher level cognition, for example, disadvantageous, high-risk decision making [45]. A study of Navy Seals during the highly stressful training period (i.e., Hell Week) demonstrated that caffeine improved vigilance and speeded reaction time [36,37]. However, it was less effective for more complex cognitive tasks, such as working memory, marksmanship accuracy and time to sight the target. Other studies have noted a similar lack of efficacy for caffeine in higher cognitive tasks [46,47]. This study extends these previous findings by being the first study, of which we are aware, to show reduced motor memory consolidation with caffeine.

Although caffeine is clearly effective in increasing arousal, the studies cited above indicate that the perceived cognitive benefit of caffeine may not universally translate to objective performance. High consumers of caffeine demonstrate faster simple and choice reaction times and report positive subjective effects in response to caffeine administration. Moderate to low users, on the other hand, do not demonstrate these enhancements [48]. Performance improvements from caffeine in some studies may thus represent a relief from withdrawal symptoms in high users. Consistent with this withdrawal hypothesis, we show no benefit to memory performance with caffeine, even in moderate consumers of caffeine (100–200 mg per day). One could also argue based on these data that this relatively low dose of caffeine actually slightly impairs the ability to learn new information (Figs 2c and 3). Such an impairment of performance, if replicated, runs counter to the general society assumption that caffeine typically benefits cognitive performance (in this case, verbal and motor memory).

6. Limitations and caveats

One limitation of this study is that only one dose of caffeine was administered. Thus, the findings should not be generalized beyond this single dose of caffeine, roughly equivalent to two–three cups of coffee. Future studies using multiple doses may show a dose-response effect on motor and verbal memory. It is possible that a group receiving either a higher or lower (than 200 mg) dose of caffeine would exhibit more optimal arousal states and relatively improved performance relative to the placebo or nap group. Similarly, future studies may wish to use multiple doses of sleep (i.e., different lengths of sleep opportunity) to also test whether a dose response relationship exists with napping for verbal memory as reported for perceptual learning [22,23]. Further, since the current study did not acquire plasma levels from administration during the actual experiment, it is possible that subjects misreported their daily caffeine intake and/or ingested caffeine on the morning of the experimental day [49]. Habitual caffeine usage has been shown to moderate performance enhancement abilities of caffeine [48]. The degree to which this caveat biases the data is lessened by the fact that subjects were randomized to their treatment group in the middle of the experimental day. Therefore, the likelihood of caffeine ingestion would be equal across groups. If anything, use of caffeine by some subjects on the day of the experiment should have minimized treatment effects, and thus minimize differences between our groups. If this were true, we may actually underestimate the differences between naps and caffeine here.

One possible explanation for the motor decrements reported in the present study is that caffeine impairs local motor movements. Typically, it is thought that at least 5 mg/kg is needed to produce hand tremors. But a few studies have found even lower doses can induce tremors [50,51]. The moderate dose of 200 mg may also have caused a global overstimulation to the nervous system, even without overt hand tremors, which impaired performance. Although subjects reported typically consuming 100–200 mg of caffeine a day, this is likely absorbed through a caffeinated beverage. Oral administration of the pill-like substance may be consumed suddenly than sipping a cup of coffee. This heightened increase in arousal may have impaired learning, as the Verhics-Dodson law states that performance is poor at high and low arousal states.

It is possible that listening to a book on tape during the time interval reserved for sleep in the napping group may have caused interference in the caffeine and placebo groups for the verbal memory task. However, the fact that the book on tape started approximately 2 h after the morning test session was completed may have reduced this possibility. If such interference did occur, the caffeine group appeared to suffer more from interference than the placebo group, since the caffeine group performed marginally worse than the placebo group for the 20-min Recall of Word List 2. Finally, the choice of a language-based activity for this control period has the advantage of preventing (or at least reducing) rehearsal of Word List 1 in the drug groups, which would have potentially increased memory consolidation in those groups independent of the drug intervention.

7. Conclusion

Overall, a daytime nap generally improved performance across three different learning paradigms, while caffeine impaired (or at least did not benefit) performance. We hypothesize that the pattern of results demonstrated by the caffeine group may be explained by the relative level of explicit information in each memory task. The three tasks, perceptual learning, procedural motor skill, and verbal
memory, each have varying levels of explicit information involved in learning. The perceptual learning task involves the least amount of explicit material, as demonstrated by the high degree of specificity shown in performance profiles [30] and no conscious access to learning or deterioration [22]. The motor task, although procedural, shows a strong explicit component, in that explicit sequence knowledge has been shown to modify off-line consolidation [52]. Also, subjects report consciously practicing the specific sequence before training and test [53]. The verbal task is by nature an explicit task in which subjects must consciously hold on to individual test words for later recall.

Explicit memory in tasks has been shown to be related to the degree that the task engages the hippocampus [54]. Sleep-dependent memory improvement in hippocampal-related tasks appears to be related to SWS [55]. In particular, Cai and Born have demonstrated that low acetylcholine during SWS is important for explicit verbal memory [56], but not implicit memory. Acetylcholine naturally decreases during sleep, whereas caffeine has been shown to increase hippocampal acetylcholine via antagonism of local adenosine A1 receptors [57]. This increase in hippocampal acetylcholine by caffeine may block the consolidation process by blocking replay of new memories. Consistent with this conceptualization, we found that the greater the explicit component of each task, the worse the caffeine group performed.

Recent attention to the importance of overnight sleep for a variety of health and cognitive domains has demonstrated that no complete pharmacological alternative to a good night’s rest has been discovered. The present findings suggest that caffeine, the most common pharmacological intervention for sleepiness, may not be an adequate substitute for the memory enhancements of daytime sleep, either.

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References

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Chapter 5

REM, not incubation, improves creativity by priming associative networks

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The hypothesized role of rapid eye movement (REM) sleep, which is rich in dreams, in the formation of new associations, has remained anecdotal. We examined the role of REM on creative problem solving, with the Remote Associates Test (RAT). Using a nap paradigm, we manipulated various conditions of prior exposure to elements of a creative problem. Compared with quiet rest and nonREM sleep, REM enhanced the formation of associative networks and the integration of unassociated information. Furthermore, these REM sleep benefits were not the result of an improved memory for the primed items. This study shows that compared with quiet rest and nonREM sleep, REM enhances the integration of unassociated information for creative problem solving, a process, we hypothesize, that is facilitated by cholinergic and noradrenergic neuromodulation during REM sleep.

The night before Easter Sunday of that year I awoke, turned on the light, and jotted down a few notes on a tiny slip of paper. Then I fell asleep again. It occurred to me at 6 o’clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. The next night, at 3 o’clock, the idea returned. It was the design of an experiment to determine whether or not the hypothesis of chemical transmission that I had uttered 17 years ago was correct. I got up immediately, went to the laboratory, and performed a single experiment on a frog’s heart according to the nocturnal design.

Otto Loewi, 1938

German, Nobel laureate for his work on the chemical transmission of nerve impulses.

Creativity has been defined as “the forming of associative elements into new combinations which either meet specified requirements or are in some way useful” (1). It has been further proposed that creative problem solving is reached in four successive phases: first, intense but unsuccessful confrontations with the elements of the problem; second, a decision to put the problem aside; third, a dormant period with no further conscious work on the problem, e.g., incubation; and finally, a “flash of insight” in which the solution suddenly enters consciousness while the individual is dreaming or engaged in idle thought (2–4). Evidence for the role of these phases in creative problem solving (e.g., a dormant period or incubation), however, is inconsistent (5–7). Yet, it has been long hypothesized that creative problem solving is enhanced by states of mind, such as sleep or quiet reflection, which foster insights. Furthermore, several famous anecdotes attribute creative revelations to dreaming in particular, ranging from musical compositions to insightful advances in scientific discovery (8).

Evidence for the role of sleep in creative problem solving has been suggested by prior research, but the most critical questions about this effect remain unanswered. First, sleep appears to enhance creative and associative memory processing compared with wake, but the underlying mechanisms, such as sleep stages, have not been explored (9–12). The seminal article by Wagner and colleagues (12) suggested that sleep might facilitate “cognitive flexibility” and lead to increased occurrences of insight; however, information about the operative sleep stage was not provided. Second, no study has demonstrated that REM [a potentially more facilitative state of mind than nonREM (NREM) sleep, REM, or wake] enhances creativity more than wake, NREM, or simply the passage of time (i.e., incubation) (13, 14). Circadian rhythms in the timing of testing periods may be a possible reason for the lack of difference between the REM group and wake controls. Last, although these studies suggest that exposure to the elements of a problem before sleep is necessary for insights to occur, they do not successfully distinguish between improved memory and enhanced creative processing as the cause of better performance on these associative tasks (15). In conclusion, prior studies suggest that sleep, particularly REM, may enhance the formation of associative networks and the integration of unassociated information, but no study to date has shown REM to enhance creative processing directly more than any other sleep or wake state. The present study (i) directly compared REM, NREM, and wake controls while using a nap paradigm to control for circadian effects, and (ii) probed contributions of both memory and associative processing in creative problem solving.

We compared incubation and sleep on three forms of prior exposure to the elements of a creative problem (repeated exposure, no exposure, or priming) on the Remote Associates Test (RAT) (Fig. 1). Using a nap paradigm allowed us specifically to compare sleep with or without REM with incubation. In the creativity task (RAT), subjects are required to produce a word that is associated with three test words that are seemingly unrelated to each other (1). The exposure conditions were designed to access three different methods for creative problem solving. First, the repeated-exposure condition examined the role of incubation on creative problem solving. Second, the priming condition examined whether stimulation of information nodes by an unrelated source can increase solutions to creative problems. Last, the no-exposure condition examined whether general creative problem solving can be enhanced with repetition of the same type of task.

We hypothesized that (i) incubation alone would increase creative associations in the repeated condition; (ii) sleep, specifically REM, is required for associating information primed in an unrelated task to the solutions for a creativity task; and (iii) creative problem solving requires prior exposure to problem-related information, such that no benefit would be seen for items in the no-exposure condition. To reduce interference effects that occur during normal waking, a quiet rest group with EEG monitoring was used instead of uncontrolled wake or sleep deprivation groups.

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Improvements

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of sleep on cognition (17–20), we find that it was not the quantity (i.e., TST), but the quality (i.e., specific sleep stages) that led to improved performance. These results suggest that REM enhanced the formation of associative networks and the integration of unassociated information, compared with quiet rest and NREM sleep.

Creative Problem Solving Requires Prior Exposure. Because daytime sleep has been shown to increase alertness and improve a range of cognitive functions (perceptual, verbal, and motor learning; declarative and implicit memory) (18, 21–24), we tested whether sleep or quiet rest might enhance general creativity on new RAT items. Baseline assessments were measured on the morning RAT. In the PM session, subjects were tested on new RAT items. Surprisingly, no group (NREM, REM, quiet rest) differences were found on the new RAT items (P = 0.261 1-way ANOVA) (Fig. 5), and no improvement in PM performance above baseline was observed in the three groups (CI 95%, −13.0 to 41.2%; CI 95%, −16.0 to 35.4%; and CI 195%, −15.4 to 51.0% for the REM, NREM, and rest groups, respectively). Although daytime sleep has been shown to improve performance on some cognitive tasks and to increase alertness and restore homeostatic drive, neither NREM nor REM sleep improved general creative problem solving in the absence of prior exposure (e.g., priming).

REM improvements in Creative Problem Solving Are Not Caused by Improved Memory. Previous studies have shown that sleep facilitates the retention of declarative memories (25–27). We, therefore, examined whether enhancement on the RAT items after priming and REM sleep was caused by memory of the answers in the priming task. To address memory, recognition and cued recall were assessed for answers to the morning analogies during the afternoon session. The process dissociation procedure (28) was also used to investigate how sleep may act on implicit and explicit memory processes.

Surprisingly, yet consistent with the incubation findings, no difference was observed among the three groups (NREM, REM, and quiet rest) for any of the memory measures, including recognition (P = 0.283, 1-way ANOVA), cued recall (P = 0.353, 1-way ANOVA), explicit (P = 0.435, 1-way ANOVA), and implicit (P = 0.229, 1-way ANOVA). Interestingly, all three groups (rest, NREM naps, REM naps) had >90% correct on the recognition test, suggesting that all groups formed memories of the answers from the morning analogies, i.e., priming. NREM sleep, REM sleep, and quiet rest groups were indistinguishable on the memory measures (recognition (P = 0.28, 1-way ANOVA), cued recall (P = 0.35, 1-way ANOVA), explicit (P = 0.43, 1-way ANOVA), and implicit (P = 0.22, 1-way ANOVA) memory). Performance on these memory measures was not correlated with performance on the primed RAT items (Fig. 6). Importantly, although all groups had similar memory for primed answers, only subjects with REM sleep promoted generalization of the analogy answers to new and useful solutions on an unrelated creative problem-solving task.

Discussion

Here, we report that REM sleep can improve creative problem solving. We found that: (i) the passage of time (i.e., incubation period) improves problem solving for previously exposed items, and this was independent of the sleep condition; (ii) sleep enhanced creative problem solving for items that were primed before sleep, but this was only true for naps that included REM sleep; (iii) REM sleep improvements in creative problem solving
are not the result of selective improvements in memory; and (v) general problem-solving abilities were not improved in wake or sleep conditions. These findings have important implications for how sleep, specifically REM sleep, might foster the formation of associative networks.

A longstanding, critical issue in sleep and cognition research is that REM sleep, which is known to improve behavioral performance, are the result of sleep-specific enhancement or reduction of interference. Experiences during waking have been shown to interfere with memory consolidation (29). Thus, performance benefits observed after sleep may be the result of lack of interference. For example, a recent study found that a quiet wake interval provided benefits for auditory tone sequence learning similar to those from a sleep interval, and both were better than the active wake interval (30). Our methods control for interference effects by comparing sleep periods with quiet rest periods. Subjects were relaxed on a recliner, with polysomnographic monitoring, and they listen to instrumental music of their choice for a time interval equal to the nap. By controlling for verbal input, we can be confident that performance across sleep and wake groups was not caused by a difference in verbal interference during the nap, but specifically by processes occurring during incubation.

Current models for why we sleep posit an important role of sleep in memory consolidation (31–34). While controlling for circadian effects, the present study found that sleep specifically enhanced the associative network for primed solutions but did not improve memory consolidation. Although other studies have reported that sleep enhances explicit verbal memory (22, 35), our memory measures were not comparable with these recall tasks because exposure to the verbal material was primed and not explicitly presented. Furthermore, not only were NREM sleep, REM sleep, and quiet rest groups indistinguishable on the memory measures, performance on those memory measures was not correlated with performance on the primed RAT items. This indicates that the improvement on the primed RAT was not a consequence of the REM sleep group simply remembering the primed words better than the other groups. These results are consistent with the previous finding that memory strength of previously encountered insight problems is not directly related to the solution acquisition to those problems (36).

The results support the hypothesis that the brain is subconsciously spreading activation of previously activated nodes. Prior literature suggests that during a “dormant period” between two active encounters with a problem, the memory trace of a target item, and the progression of this target through other relevant stored information generate spreading activation through a network (16). For example, by priming the solution SWEET before sleep, the SWEET node is activated, and during subsequent REM sleep, the associative nodes (in this case HEART, SIXTEEN, COOKIE) are more likely to be activated and increased above threshold. Therefore, when the three words that were previously unrelated (HEART, SIXTEEN, COOKIE) are seen, there will be an increased probability of the node SWEET being chosen as the solution. We propose that the most optimal dormant period occurs during REM sleep, which provides the most spreading of activation.

One possible mechanism for the spreading-activation model involves cholinergic and noradrenergic neuromodulation that occurs specifically during REM sleep. During wake, higher levels of norepinephrine and acetylcholine inhibit recurrent connections in the neocortex. During REM sleep, however, high levels of acetylcholine in the hippocampus suppress feedback from the hippocampus to the neocortex, whereas lower levels of acetylcholine and norepinephrine in the neocortex could facilitate the spread of activity within neocortical areas without strong hippocampal influence (37). This is supported by behavioral evidence from amnesiacs that activation of this associative network during sleep is independent of the medial temporal lobe structures and may reflect reactivation of remote memories that are less dependent on the hippocampus (38). In this theoretical framework, REM sleep would allow neocortical structures to reorganize associative hierarchies, in which information from the hippocampus would be reinterpreted in relation to previous semantic representations or nodes.

We propose that REM sleep is important for assimilating new information into past experience to create a richer network of associations for future use. Fluid interpretation is a hallmark of a creative mind, as fluid words play a prominent role in the abstraction of concepts that led to the solving of neurochemical transmission or the structure of the benzene ring. These findings on the role of REM sleep in creative problem solving underscore the Nobel Laureate Friedrich A. Kekule’s recommendation: “Let us learn to dream.”

**Experimental Procedures**

General procedures are outlined in this section. Deviations from this procedure are described in each section.

**Subjects.** A total of 77 native English speakers between the ages of 18 and 35 with no personal history of neurological, psychological, or other chronic illness gave informed consent to participate in the experiment, which was approved by the Institutional Review Board of the University of California at San Diego. Subjects were required to sleep an average of 8 h per night for the 5 days leading up to the experimental day and at least 6.5 h the night before the test day. Subjects filled out sleep diaries and wore actigraphs 5–7 days before testing as subjective and objective measures of sleep-wake activity. Subjects were restricted from consuming caffeine and alcohol 24 h before and during the experiment day.

**Materials.** RAT is a paper-and-pencil task adapted from Mednick (1). Each RAT item contains a triplet of words presented horizontally along with a blank space. Each item requires the subject to combine or relate the three words drawn from mutually remote associative clusters (e.g., COOKIES, SIXTEEN, HEART...). The subject is required to find a fourth word that could serve as an associative link between these three words. The answer to this item is SWEET (cookies are sweet, sweet sixteen, sweetheart). The three test words HEART, SIXTEEN, COOKIE are associated with the solution SWEET by formation of a compound (sweetheart), by a syntactic association (sweet sixteen), and by a semantic relationship (cookies are sweet). Thus, reaching a solution requires “creative thought” because the first, most highly probable associate to each of the items is related to the solution, so the solver must think of more distantly related information to connect the three words. Performance on the RAT correlates reliably with other established insight problems (19).

Subjects were read the instructions aloud and given four practice items to ensure understanding of the task. The score was calculated as the proportion of items answered correctly. Subjects completed two versions of the RAT, one in the morning and one in the afternoon. The two versions were counterbalanced across sessions. In the morning session, subjects completed the AM RAT, and AM scores were used as baseline.

**Analogies.** Analogies (e.g., FAST/SLOW as HARD/...), were administered in the AM session. The first letter of each answer was given. Half of the analogy answers served as primes for the answers to the RAT administered during the PM session. There was no time limit for completing the analogies. The mean log HAL word frequency (40) for all words used in the analogies was 1.58 (SD = 0.73). The mean word length was 5.38 letters (SD = 1.75).

**General Method.** All subjects were tested on the RAT twice in 1 day (Fig. 1). At 0900, subjects were administered the RAT followed by the analogies. Subjects returned at 1300, at which time they were randomly assigned to a nap or a quiet rest group. Subjects in the nap group took a polysomnographically recorded (PSG) nap (30 min of sleep maximum or up to 2 h in bed), whereas those in the rest condition listened to instrumental music with PSG monitoring for 90 min. At 1630, subjects returned for testing of the afternoon RAT.

Experiment 1. RAT. Subjects completed 30 RAT items. They were given 40 min to complete all of their responses. Analyses. Two versions with 20 analogies each were administered in the AM session. Fifteen analogies in the AM had the same answers as 15 items in the PM RAT.

Study procedure. In the morning session, subjects completed the AM RAT and then filled in responses to 30 analogies. In the PM RAT, 15 of the items were primed by the AM analogies. The other 15 PM RAT items were unprimed. A total of 25 subjects, REM(n = 10), NREM(n = 6), and rest(n = 9), participated in experiment 1.

Experiment 2. RAT. Two shortened versions of the RAT were used, each with 15 items. These versions were created by deleting Version A of the RAT into odd and even items. The two versions were counterbalanced across sessions. Subjects were given 20 min to complete all of their responses. The morning RAT-5 was used as a baseline performance measure. In the afternoon session, two RAT-5 forms were administered. One form was the same as the form of the morning (i.e., unused RAT-5), and the other form had the 15 answers cues from the morning analogies (i.e., used RAT-5).

Analyses. Subjects completed 75 analogies. Fifteen correct answers were primed for the afternoon used RAT-5. The remaining 60 analogy answers were used in the afternoon session to test for memory retention with three distinct methods. Twenty correct answers were tested on a recognition test, 20 correct answers were tested on an exclusion test (see below for test description). Inclusion and exclusion tests. A modified version of the process-dissociation procedure (15) was used to dissociate between explicit and implicit processes for verbal learning. For the inclusion test, subjects were given 20 item completions and asked to complete with words they recalled from the answers to the morning analogies. If they could not recall the word, they were asked to fill in the stem with the first word that came to mind. For the exclusion test, subjects were also given 20 item completions and asked to complete with words that were NOT answers to the morning analogies. If they could not recall the word, they were to fill in the stem with the first word that came to mind. The 40 words were counterbalanced across the inclusion–exclusion test and condition. Explicit memory was calculated as inclusion–exclusion. Implicit memory was calculated as exclusion[1]–(inclusion + exclusion) (28).

Statistical analyses. Three tests of percentage improvement on RAT performance (repeated exposure, primed exposure, no exposure) were examined with a 2-tailed, 1-way ANOVA by using three levels of the variable Group (REM, NREM, quiet rest). Percentage improvement was calculated as (number of correct responses on afternoon RAT – number of correct responses on morning baseline RAT)/number of correct responses on morning baseline RAT. Percentage improvement was computed for each individual and then averaged across subjects for each group. CI values were set at 95%, with a 2-tailed probability to examine whether percentage improvement on the afternoon RAT differed from 0 (i.e., no improvement). To examine the effect of specific sleep stages on creative problem solving, we categorized data on the presence or absence of REM sleep as indicated by PSG. This segregated the data into naps with REM, NREM, and quiet rest groups. Performance for new and repeat exposure was calculated from experiments 1 and 2, respectively. There were no differences between experiments 1 and 2 for performance in primed-exposure RAT items [F = 0.26, 2-way ANOVA (Experiment x Group)], so percent improvement for each subject was collapsed across experiments 1 and 2.

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GENERAL DISCUSSION

This body of work strongly supports that sleep is important for memory consolidation in both humans and rodents. In Chapter 1, we experimentally addressed the criticisms of sleep deprivation paradigm in rodents. This was particularly important because behavioral findings in rodents on sleep and memory have been done almost exclusively with a sleep deprivation paradigm. While many have critiqued this procedure for possible confounds, up until now, no one has experimentally asked if the deprivation method (i.e., gentle handling) produces deficits unrelated to sleep. This is the first direct evidence that this deprivation procedure in induces deficits independent of sleep. These results raise concern about using deprivation procedures in studying the relationship between memory and naturalistic sleep and awake cycles. These data caution other researchers when using this popular procedure and encourage using a more naturalistic approach. In Chapter 2, we detailed a novel rodent paradigm to examine consolidation processes that occur across normal sleep and awake cycles. This study carefully controlled for both time passage and circadian rhythm without using a deprivation paradigm. We found sleep to selectively enhance hippocampus-dependent memory in mice. While this has been predicted by the neural replay literature, the link between sleep and improvement in behavior has never been demonstrated until now. In exploiting different approaches in answering the same question, we studied the relationship between pharmacologically induced sleep and memory. We found that therapeutic doses of zolpidem (0.01-0.5 mg/kg) did not affect fear acquisition or consolidation of fear memory. Consistent with previous findings (Depoortere et al., 1986), we found that 8mg/kg of zolpidem induced deep sleep. Next, we explored the effects of pharmacologically induced deep sleep and memory consolidation. Specifically, we wanted to know if a “mouse nap” (i.e. sleep during a non-circadian time for sleep)
would selectively enhance contextual memory. If so, this would further give evidence that consolidation for contextual memory requires sleep rather than just co-occurring at the same circadian time as sleep. In this study, we trained mice on fear conditioning an hour prior to their main awake or sleep phase and immediately administered 8mg/kg of zolpidem (i.e. induced deep sleep) or saline. We then tested them 12 hours later at the end of either their awake or sleep phase. We found that inducing deep sleep during the awake phase (i.e. zolpidem induced nap) increased context memory compared to saline controls with an awake phase between training and testing. However, inducing deep sleep during the sleep phase did not further improve contextual fear memory. This gives further evidence that these enhancements are specific to sleep and not a different process that occurs at the same circadian time as sleep.

In Chapter 4, we used a nap paradigm to investigate the role of sleep on different memory domains in humans. Previous studies have mainly used nocturnal sleep paradigms, which may be confounded by deprivation, circadian or homeostatic concerns. This study controlled for these confounds by simply adding an extra bout of sleep in the middle of the day. We found that sleep is important for human memory consolidation, including hippocampus-dependent memory. Importantly, we found that the nap benefits were specific to consolidation and were not a result of improved acquisition or retrieval processes. Lastly, in Chapter 5, we explored how sleep facilitates the reorganization of representations. Using a nap paradigm, we found the REM sleep was critical for integrating new information with prior associative networks.

**SWS vs REM?**

While much of the prior studies have dichotomized SWS and REM to contribute to either declarative or procedural memory, respectively, results from both our rodent and human studies suggest that the interplay of SWS and REM may differentially
facilitate a single memory. SWS may be the brain state in which new information is laid down in hippocampus as well as communicated to cortex. REM may be the state in which new representations are reorganized and assimilated with prior associations.

One model of memory that describes the interplay of SWS and REM (Buzsaki, 1989; Hasselmo, 1999) posits that high levels of acetylcholine (ACh) in the hippocampus during active wake allow for encoding of new memories, while low levels of ACh in the hippocampus during quiet rest and SWS facilitate both cellular and systems consolidation of these memory traces. During active wake information coded by neocortical structures flows to the hippocampus via the entorhinal cortex and dentate gyrus. Here, synaptic modification forms an intermediate-term representation binding together different elements of an episodic memory. During active wake, high levels of ACh in hippocampus inhibit systems consolidation by suppressing output connections from hippocampus (i.e. CA3 to CA1 as well as CA1 outputs). Conversely, during quiet wake or SWS, low levels of ACh enhance these connections and the flow of information out of the hippocampus to cortex. Sharp waves flow through region CA1 to entorhinal cortex and then to cortex, enabling the stabilization of memory in hippocampal regions as well as cortex. During REM, levels of ACh in hippocampus are high, similar to active wake, suppressing the dialogue between hippocampus and cortex. This suppression of information from hippocampus may facilitate the integration and reorganization of new information in prior association cortices without interference from other structures (i.e. hippocampus). This might underlie modification of declarative memory within circuits of association cortex. Memories likely need both brain states to integrate and assimilate the information to create a richer network of experiences.

Our human and animal data is consistent with this model of memory consolidation posed by Buzsaki (1989) and Hasselmo (1999). In humans, we found naps
improved consolidation of verbal retention compared to caffeine and an equal duration of a waking period. According to this model, the relative higher levels of ACh in the caffeine and awake groups may have suppressed the dialogue between hippocampus and cortex, therefore not facilitating hippocampal consolidation. We also found that pharmacologically induced deep sleep (i.e. increased SWS) improved contextual fear memory in mice. As proposed by Buzsaki (1989) and Hasselmo (1999), consolidation processes of hippocampal memories, such as reactivation, may have been enhanced due to the decreased levels of ACh during deep sleep, such as SWS. Furthermore, we found REM sleep to facilitate the integration of new information with prior representations, possibly due to the increased levels of ACh, which may be suppressing hippocampal dialogue. This suppression of information from hippocampus may allow for cortical structures to redistribute and reorganize new information without interference from hippocampus.

*Cellular or systems consolidation?*

Of the two different stages of memory consolidation (cellular and systems) much of the neural replay literature has suggested sleep to facilitate systems consolidation, a long protracted process that is typically thought to occur on the order of weeks to months. However, our data, along with many other studies, clearly show that sleep enhances consolidation within hours of the first day in both humans and mice. We observed that naps within a few hours of training benefited verbal memory. We also found sleep within the first 12 hours, both natural and pharmacologically induced, enhanced contextual fear memory. These time-scales are well within those of cellular consolidation. Additionally, neural replay, one of the leading theories of systems consolidation, has typically been observed to occur most robustly and reliably within the first few hours after the experience (e.g. maze running), and the neural signals dissipate
across several hours to days after the experience (Ji & Wilson, 2007; Poe, Nitz, McNaughton, & Barnes, 2000). Similarly, in Donald Hebb’s famous theory on synaptic plasticity, his description of replay or reactivation fits within the same time course as cellular consolidation:

“Let us assume that the persistence or repetition of a reverberatory activity (or "trace") tends to induce lasting cellular changes that add to its stability…. When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased.” (Hebb, 1949)

Similar to the short time scale of neural replay in rodents, recent evidence suggests that something akin to neural replay also occurs in humans. Cuing recently formed odor-associated memories by odor re-exposure during SWS -- but not during REM sleep -- prompted hippocampal activation (as measured by fMRI) and resulted in less forgetting after sleep than compared to controls (Rasch et al., 2007). This result is consistent with the notion that reactivation of newly encoded hippocampal representations occurs during SWS, which may lead to systems consolidation. In a conceptually related study, hippocampal areas that were activated (as measured by regional cerebral blood flow) during route learning in a virtual town (a hippocampus-dependent, spatial learning task) were activated again during subsequent SWS (Peigneux et al., 2004). Moreover, the degree of activation during SWS correlated with performance on the task the next day.

In both of these studies, the hippocampal reactivation (perhaps reflective of hippocampo-cortical dialogue) occurred within hours of the learning episode, a time course of consolidation ordinarily associated with cellular consolidation. The timing observed in these studies is not unlike that observed in a neuroimaging study in which hippocampal activity decreased, and cortical activity increased, over a period as short as
24 hours (Takashima et al., 2009). Subjects in that study memorized two sets of face-location stimuli, one studied 24 hours before the memory test (old memories) and the other studied 15 minutes before the memory test (new memories). To control for differences in memory strength, they compared activity for high-confidence hits associated with the old and new memories and found that hippocampal activity decreased and neocortical activity increased over the course of 24 hours. In addition, the connectivity between the hippocampus and the neocortical regions decreased, whereas cortico-cortical connectivity increased (all over the course of only 24 hours). Results like these suggest that the process of systems consolidation can occur very quickly.

More direct evidence reinforces the possibility that the processes underlying cellular and systems consolidation may be more interlinked than previously thought. For example, α-CaMKII – a kinase known to be essential for the induction of hippocampal LTP and cellular consolidation – also plays a critical role in the consolidation of memory traces in cortical networks. In this study, mutant mice with reduced levels of α-CaMKII in the cortex had normal contextual fear memory after 1 day but not after 10 days (Frankland, O'Brien, Ohno, Kirkwood, & Silva, 2001). These findings suggest that similar mechanisms that are required for cellular consolidation may also be required for systems consolidation.

These data taken together strongly question the time-scale and distinctiveness of cellular and systems consolidation. It has been long thought that cellular consolidation occurs on the order of minutes to hours, while systems consolidation unfolds on the order of weeks to years and that these systems operate independently. However, in light of these data, we must reconsider how these two systems operate.
Not so long ago, the notion that sleep was essential for memory consolidation was more a wishful dream than an evidence-based reality. In the last decade, the literature has emerged with behavioral, pharmacological and cellular evidence outlining the importance of sleep for the consolidation processes to unfold. It is possible that sleep is the optimal state for both cellular and systems consolidation.
REFERENCES


