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## The role of napping on memory consolidation in preschool children

Laura Kurdziel  
*Neuroscience and Behavior*

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THE ROLE OF NAPPING ON MEMORY CONSOLIDATION IN PRESCHOOL  
CHILDREN

A Dissertation Presented

by

LAURA B. F. KURDZIEL

Submitted to the Graduate School of the  
University of Massachusetts Amherst in partial fulfillment  
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2014

Neuroscience and Behavior Program

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ABSTRACT

THE ROLE OF NAPPING ON MEMORY CONSOLIDATION IN PRESCHOOL  
CHILDREN

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Nocturnal sleep has been shown to benefit memory in adults and children. During the preschool age range (~3-5 years), the distribution of sleep across the 24-hour period changes dramatically. Children transition from biphasic sleep patterns (a nap in addition to overnight sleep) to a monophasic sleep pattern (only overnight sleep). In addition, early childhood is a time of neuronal plasticity and pronounced acquisition of new information. This dissertation sought to examine the relationship between daytime napping and memory consolidation in preschool-aged children during this transitional time. Children were taught either a declarative or an emotional task in the morning, and memory was probed following a nap and following an equivalent period of wake. Memory was also examined 24-hours later in the morning after overnight sleep. Overall, memory for both declarative and emotional information was shown to benefit from the nap. In both experiments, napping protected encoded information, whereas wake-promotion during the day led to an approximate 10% reduction in memory accuracy the following morning. Performance on the declarative memory task was associated with sleep spindles in the nap, whereas performance on the emotional memory task was

related to nap slow wave activity. The results of this dissertation indicate that napping is important for memory consolidation of newly learned information in preschool-aged children. As such, this work supports the continued practice of nap promotion in preschool classrooms.

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## CHAPTER 1

### BACKGROUND AND SIGNIFICANCE

Sleep is defined as a reversible period of behavioral quiescence with a high arousal threshold. Sleep is also considered to be a state of vulnerability in the wild and a state in which reproduction and nourishment are not possible. Since sleep-like states are observed across the animal kingdom despite these vulnerabilities, it is likely that sleep is necessary and adaptive for survival (Siegel, 2009). Two theories that dominate the literature as to the function of sleep are that sleep is restorative, allowing brain metabolism to return to homeostasis (Benington & Heller, 1995), and that sleep enhances memory consolidation (Diekelmann & Born, 2010). Importantly, these theories are not mutually exclusive and both of these functions are necessary for healthy development.

Sleep patterns change throughout the lifespan. Human infants sleep between 10 and 18 hours a day (Williams, Zimmerman & Bell, 2013). Infant sleep is polyphasic, meaning that sleep is distributed across multiple sleep bouts in a 24-hour period (Meltzer & Mindell, 2006). By the time children are toddlers, most have consolidated sleep to 3 bouts – an overnight period, and two mid-day naps (Iglowstein, Jenni, Molinari & Largo, 2003). During the preschool years (~3-5 years of age), children still sleep between 9 and 15 hours across the day, but they become monophasic, consolidating sleep to one nocturnal bout (Weissbluth, 1995; Thorleifsdottir, Björnsson, Benediktsdottir, Gislason & Kristbjarnarson, 2002). Throughout the remainder of childhood and adolescence, nocturnal sleep time decreases (Iglowstein, et al., 2003) reaching approximately 7 hours by early adulthood (National Sleep Foundation, 2010).

## Sleep Architecture

Sleep is not a static behavioral state. Human sleep can be divided into two states, Rapid Eye Movement (REM) sleep, and non-REM (nREM) sleep. Non-REM sleep can be further subdivided into three sleep stages: nREM1, nREM2 and nREM3. Each sleep stage is physiologically unique and can be characterized through polysomnography (PSG), a montage of electroencephalogram (EEG) waveforms, muscle activity measured by electromyogram (EMG), and eye movements as measured by electrooculogram (EOG; Carskadon & Dement, 2005).

Non-REM1 is differentiated by a slowing of the EEG waveforms relative to the awake brain; alpha waves, which are seen when an individual is awake but tired, decrease while theta waves, which are lower in frequency, increase. In addition, slow rolling eye movements are present. The most prevalent sleep stage over the course of the night is nREM2. Non-REM2 is identified by two principle physiological markers: K-complexes and sleep spindles. K-complexes are large amplitude negative deflections followed immediately by a positive deflection in the EEG (Iber, Ancoli-Israel, Chesson & Quan, 2007). Sleep spindles are high frequency theta bursts indicative of thalamocortical, or hippocampocortical interaction (see review: De Gennaro & Ferrara, 2003). Non-REM3 is more commonly referred to as slow wave sleep (SWS) due to its characteristic slow, large amplitude EEG waves. REM is also known as paradoxical sleep because the EEG waves look very similar to wake, but has characteristic rapid eye movements in the EOG and muscle atonia is observable in the EMG (Iber, et al., 2007).

A bout of overnight sleep is typically composed of multiple REM/nREM sleep cycles. Throughout infancy, the sleep cycle is typically 50 – 60 minutes in length (Ficca,

Fagiolo & Salzarulo, 2000). In a mature adult, the average period of the sleep cycle is 90 minutes (Carskadon & Dement, 2005).

### The Memory Function of Sleep

As demonstrated by PSG, brain activity is dynamic during sleep, suggesting that sleep also has dynamic functions. Of particular interest in this dissertation is the memory function of sleep. A number of studies suggest that memories are consolidated over sleep (see review: Stickgold, 2005).

Memory consolidation, or the process through which a memory trace becomes more stable and less vulnerable to forgetting or interference, is greatest over sleep (Stickgold, 2005; Walker, 2005). Sleep-dependent memory consolidation (SDC) involves cellular and molecular changes, as well as changes at the systems level, to stabilize a particular memory for long-term recall and to integrate the memory into other existing memories. In one of the earliest studies of memory consolidation over sleep, Jenkins and Dallenback (1924) found that memory for nonsense syllables was greater following bouts of sleep than following bouts of wake. Since then, the role of sleep on declarative memory has been more thoroughly investigated, and many studies have replicated the finding of improved recall following sleep compared to recall following wake. For example, when individuals were taught a list of semantically unrelated word-pairs either in the morning (wake group) or in the evening (sleep group), recall accuracy was significantly greater 12 hours later when the intervening interval contained sleep (Donohue & Spencer, 2011; Wilson, Baran, Pace-Schott, Ivry & Spencer, 2012). These results suggest that during sleep, memories are actively consolidated, leading to reduced forgetting and more long-term retention of the encoded information.

There are alternative interpretations for the observed differences in recall following intervals of sleep and wake. One such argument is that sleep is simply a time in which no new information is being acquired; therefore interference from other stimuli is minimal. Across wake, we are constantly exposed to new and potentially interfering information. Sleep may therefore play only a passive role in improved memory recall by protecting information from this interference.

To the contrary, a number of studies suggest that sleep plays an active role in memory processing. Through sleep, synaptic connections are strengthened and stabilized leading to improved subsequent memory. One way of investigating whether sleep actively consolidates memory is to challenge a new memory with interfering information following sleep or following an equivalent period of wake. If sleep actively strengthens the memory, then learning interfering information following a bout sleep should not affect recall of the original memory. However, if sleep simply passively protects memory, then interfering information should be equally as likely to disrupt the original memory following sleep or wake. To examine this experimentally, participants first learn pairs of stimuli (A-B), (e.g. word pairs: Ellenbogen, Hulbert, Stickgold, Dinges & Thompson-Schill, 2006; pairs of spatial locations: Diekelmann, Büchel, Born & Rasch, 2011), and then following a period of wake or with sleep, participants learn new associations (A-C) as a mechanism of interference. Recall of the original pairs (A-B) is greater when interference followed sleep compared to following wake. These results suggest that sleep stabilizes and protects the memory from subsequent interference, and support that sleep plays an active role in memory consolidation.



Additionally, in support of SDC being an active process, over-sleep changes in memory can be associated with a particular sleep stage. If sleep passively protects information from interference, no one sleep stage should be more related with memory performance than any other. However, the consolidation of different types of memory are associated with different sleep stages. For example, SDC of declarative memory is often associated with time spent in SWS (see review: Gais & Born, 2004). A study by Peigneux and colleagues (2004) demonstrates the relationship between SWS and a hippocampal-dependent declarative memory task. Healthy young adult males learned to navigate through a virtual maze, following which their sleep physiology and neuronal activity were recorded using PSG and positron emission tomography respectively. The hippocampal regions most active during the learning of maze navigation *prior to sleep* were reactivated primarily during SWS. This reactivation was found to a much lesser extent in other sleep stages. No such reactivation was observed in a group that did not sleep but rested quietly. Most importantly, *post-sleep* memory recall was significantly correlated with hippocampal activation during sleep; the more neuronal replay that occurred during SWS, the better the information was remembered later (Peigneux, et al., 2004). This suggests that neuronal activation during SWS actively leads to consolidation of declarative memories.

Another argument against the idea that changes in memory across sleep reflect consolidation is that such changes may instead reflect circadian influences on performance. However, shorter delay intervals have been used so that recall can be probed at the identical circadian time for both the wake and sleep groups. Using a word-pair learning task, Lahl and colleagues (2008) demonstrated that a 60-minute nap was

sufficient to increase memory recall compared to a similar period of wake. Encoding and recall took place at the same time of day in the sleep and wake groups. All together, the literature suggests that sleep protects or enhances memory in healthy young adults whereas wakefulness leads to forgetting.

### Sleep-Dependent Memory Consolidation in Children

The aim of this dissertation is to examine SDC in preschool-aged children. Specifically, SDC was examined across a mid-day nap. It is important to determine the function of the nap during this time period. Governmental pressures to improve children's academic success have lead to increased curriculum demands even within preschool classrooms. As a result, naptime is becoming devalued. To date, no scientific research has been conducted to assess whether a nap benefits learning in preschool-aged children.

The academic functions of the preschool nap are particularly relevant given that early childhood is a time of neuronal plasticity and pronounced acquisition of new information. A peak in synapse formation and dendritic arborization within the prefrontal cortex occurs during the preschool years (Casey, Tottenham, Liston & Durston, 2005). The parietal and temporal cortices also mature during this time (Casey, et al., 2005). The hippocampus reaches adult-like volume within the first few years of life, although the microstructure of hippocampal regions continues to develop even beyond early childhood (Gogtay, et al., 2006). These considerable structural changes within the hippocampus and cortical regions are of particular importance to learning and memory in preschool-aged children.

During the preschool years, children also transition out of needing mid-day naps, converting to a monophasic sleep pattern. Iglowstein and colleagues (2003) showed this reduction in napping frequency in a longitudinal study of over 400 children. At 3 years of age, 50% of children are still napping, whereas only about 1% still nap by the age of 7 (Iglowstein et al., 2003). This transition to monophasic sleep during the preschool years has also been reported in other studies (e.g. Weisbluth, 1995; Jenni & Carskadon, 2007).

With the dramatic changes in sleep patterns and the maturity of relevant brain structures occurring throughout early development, SDC also changes throughout childhood. In infants, veridical memory for an artificial language was not better following a bout of sleep (Gómez, Bootzin & Nadel, 2006), whereas school-aged children (7-12 years; Henderson, Weighall, Brown & Gaskell, 2012) and adults (Tamminen, Payne, Stickgold, Wamsley & Gaskell, 2010) show a sleep-dependent improvement in veridical memory for newly learned novel-words. Although infants did not show SDC for words, they do show a greater abstraction of grammar rules with sleep (Gómez, Bootzin & Nadel, 2006), and this effect was long-lasting, suggesting consolidation of the rule (Hupback, Gómez, Bootzin & Nadel, 2009). Similarly, in school-aged children, sleep was shown to integrate newly learned information with existing lexical knowledge (Henderson, et al., 2012), an effect also observed in young adults (Tamminen, et al., 2010). Changes in SDC of procedural memory are also seen across development. In contrast to what is observed in young adults (see review: Stickgold, 2005), school-aged children do not benefit from sleep on tasks of motor learning (Fischer, Wilhelm & Born, 2007; Wilhelm, Diekelmann & Born, 2008; Prehn-

Kristensen et al., 2009), although this may be due to differences in encoding between children and adults (Wilhelm, Metzkw-Mészáros, Knapp & Born, 2012).

The role of SDC in learning for preschool children is not yet known. An understanding of SDC across the nap period, specifically, is necessary given that the role of the napping on memory has only been examined in infants (Gómez, Bootzin & Nadel, 2006; Hupback, Gómez, Bootzin & Nadel, 2009). One recent study suggests that the transition to a monophasic sleep pattern is indicative of brain maturation, in that napping was negatively correlated with performance on neurocognitive tests, regardless of age (Lam, Mahone, Mason & Scharf, 2011). While this supports that sleep pattern changes are paralleled by neuronal maturation, Lam and colleagues do not examine changes in memory consolidation across this period.

My dissertation uses two experiments to examine the role of napping on memory consolidation in preschool children. In chapter 2, I investigated whether napping promotes the consolidation of declarative memories in preschool-aged children compared to an equivalent period of wake, and whether the sleep physiology of the nap was related to this benefit. In chapter 3, I examined whether the benefits of napping on memory consolidation are translatable to another type of memory. Specifically, I investigated whether napping promotes the consolidation of emotional memories in preschool-aged children. In addition, I assessed whether the daytime nap affected overnight physiology, and whether these differences between the nap and wake conditions lead to differential emotional memory consolidation.

## CHAPTER 2

### THE ROLE OF NAPPING ON DECLARATIVE MEMORY CONSOLIDATION IN PRESCHOOL CHILDREN

The aim of this chapter is to assess whether preschool naps serve an academic function for declarative learning. Declarative memory is specifically targeted given that much of the directed learning that occurs in the preschool classroom is declarative in nature. Preschool children learn to label objects with an increasing vocabulary (see review: Conti-Ramsden & Durkin, 2012) and to communicate effectively with new social peers (Justice, Petscher, Schatschneider & Mashburn, 2011). Often curriculum within the preschool classroom aims to provide children with basic semantic knowledge about their surrounding world.

To assess whether naps benefit declarative learning in the preschool classroom, memory for a visuo-spatial task was examined following a nap and following an equivalent period of wake. The results of this experiment were accepted for publication by the Proceedings of the National Academy of Sciences in 2013. This paper is provided below.

## Abstract

Despite the fact that mid-day naps are characteristic of early childhood, very little is understood about the structure and function of these sleep bouts. Given that sleep benefits memory in young adults, it is possible that naps serve a similar function for young children. However, children transition from biphasic to monophasic sleep patterns in early childhood, eliminating the nap from their daily sleep schedule. As such, naps may contain mostly light sleep stages and serve little function for learning and memory during this transitional age. Importantly, lacking scientific understanding of the function of naps in early childhood, preschool classroom nap opportunities are at risk of being eliminated due to increasing curriculum demands. Here we show the first evidence that classroom naps support learning in preschool children by enhancing memories acquired earlier in the day as compared to equivalent intervals spent awake. This nap benefit is greatest for children who nap habitually, regardless of age. Importantly, performance losses when nap-deprived are not recovered during subsequent overnight sleep. Physiological recordings of the naps revealed an active role of sleep in memory consolidation, specifically through sleep spindles occurring throughout the predominately non-REM (stages 2 and 3) naps. These results suggest that distributed sleep is critical in early learning; when short-term memory stores are limited, memory consolidation must take place frequently.

## Introduction

Preschool education provides life-long benefits to physical and mental health (Ramey et al., 2000; Ramey & Ramey, 2004; Palfrey, et al., 2005; Muennig, Schweinhart, Montie & Neidell, 2009). These benefits justify state-funded preschool education in states like Oklahoma and Georgia and the petition of President Barack Obama for federal funds to support preschool education for all children (Obama, 2013). Recent research has focused on interventions to further enhance the outcomes from preschool education. For instance, studies of emotional training (Ramey et al., 2000), nutrition education (Hu et al., 2010, Sweitzer et al., 2010) and dental hygiene (Jackson et al., 2005) in preschools have led to enhanced curriculum-based learning in early education. However, with increased curriculum demands and tax-payer pressure, classroom nap opportunities are becoming devalued.

Recent studies in young adults have demonstrated sleep-dependent enhancements in learning. Such enhancements are thought to reflect sleep-dependent consolidation, a process by which memory storage and retrieval become more efficient (Stickgold & Walker, 2007). However, the characteristics of sleep patterns and the architecture of sleep vary dramatically throughout development. Between 3-5 yrs of age, total sleep time and time in 'deep' sleep stages, slow wave sleep (SWS) and rapid eye movement sleep (REM), decline significantly (Ohayon, Carskadon, Guilleminault & Vitiello, 2004; Montgomery-Downs, O'Brien, Gulliver & Gozal, 2006). Moreover, in early childhood, sleep bouts are distributed across the day. Polyphasic from birth, daytime sleep bouts are reduced to a single nap early in the preschool years, and children reach the adult-like,

monophasic pattern around 5 yrs of age due to maturational and scheduling pressures (Weissbluth, 1995).

Parallel to this period of abundant SWS-rich sleep and daytime napping, early childhood is typified by the dramatic acquisition of new information as a result of increased neuronal plasticity (Goldman-Rakic, 1987; Lenroot & Giedd, 2006). The maturation of the parietal and temporal cortices, and a peak in experience-dependent synapse formation in the prefrontal cortex occurs around the preschool age (Casey, Tottenham, Liston & Durston, 2005). Lam and colleagues (Lam, Mahone, Mason & Scharf, 2011) suggest that the elimination of the mid-day nap is a marker of this brain maturation. Specifically, they found a negative correlation between the number of times a preschool child napped during a week and performance on a battery of cognitive assessments.

Yet, whether individual sleep bouts contribute to recent memories in early childhood is unknown. Counter to what is observed in young adults, performance on a procedural memory task was not improved by overnight sleep in young (6-8 yrs; Wilhelm, Diekelmann & Born, 2008) or older children (7-13 years; Fischer, Wilhelm & Born, 2007; Prehn-Kristensen et al., 2009). Wilhelm and colleagues (2012) suggest the absence of procedural memory consolidation in children may be due to insufficient initial encoding; children with extended training did exhibit sleep-dependent improvements in motor skill.

Whether declarative memories are consolidated over mid-day naps in children has not been examined. Naps are sufficient for declarative memory consolidation in young



adults (Tucker et al., 2006). Moreover, declarative memories have been shown to benefit from nocturnal sleep in children 6-13 yrs of age (Backhaus, Hoeckesfeld, Born, Hohagen & Junghanns, 2008; Wilhelm, Diekelmann & Born, 2008; Prehn-Kristensen et al., 2009). Thus, naps may function to consolidate declarative memories throughout early life. However, given the transitional nature of naps during early childhood and the lack of physiological studies in healthy preschool age children, it is possible that naps do not contain the critical non-REM sleep (Rasch, Buchel, Gais & Born, 2007) and sleep spindles (Schabus et al., 2004) necessary for sleep-dependent consolidation of declarative memories.

To investigate whether in-class naps benefit declarative learning in preschool children, we measured changes in performance on a visuo-spatial task over a nap and an equivalent interval of wake. A visuo-spatial task was selected for three reasons. First, this task, like other declarative learning tasks, has been shown to engage the hippocampus (Postma, Kessels & van Asselen, 2008). Hippocampal-dependent tasks are subject to neural replay during sleep, a possible mechanism underlying sleep-dependent consolidation (O'Neill, Pleydell-Bouverie, Dupret & Csicsvari, 2010). Second, visuo-spatial learning has been shown to benefit from overnight sleep in young adults (Rasch, Buchel, Gais & Born, 2007). Third, the task, like the game 'Memory', is appealing to preschool children.

Children learned the task in the morning and subsequent immediate recall provided an initial measure of performance (Fig. 1). During the regularly scheduled nap opportunity in the early afternoon, children were either wake- or nap-promoted (within subjects; order counterbalanced) and delayed recall was subsequently tested. To examine

the long-term benefit of having napped, recall was tested once more the following day (24-hr recall). Additionally, we recorded polysomnography (using a 12-electrode montage) during a laboratory-based nap in a separate group of children in order to examine relevant nap physiology. We hypothesized that the preschool nap is sufficient for consolidation of newly learned information, and that sleep-dependent changes in memory would be associated with specific physiological aspects of sleep.

### Results

Forty children (31 female;  $M=49.76$  months,  $SD=8.23$ , range=36-67 months), who completed both the nap- and wake-promoted conditions, were included in the analysis. Average nap length, as documented by experimenters present in the classroom, was 77.7 mins ( $SD = 18.75$  mins).

Children performed similarly at baseline (immediate recall) in both conditions ( $t(39)=0.351$ ,  $p=0.728$ ). Yet, delayed recall was significantly greater following the nap than after equivalent time awake ( $t(38)=2.837$ ,  $p=0.007$ ; Fig. 2). As a further control for potential differences in baseline performance, a difference score, or the change in accuracy across the nap/wake period (delayed minus immediate recall), was calculated. Here too we see that significantly more items were forgotten following wake than following the nap ( $t(38)=2.457$ ,  $p=0.019$ ) showing a clear nap benefit on memory retention.

Optimal performance following the nap is unlikely to be associated with reduced fatigue or enhanced attention relative to the wake condition for two reasons. First, child-rated sleepiness did not differ for the nap and wake conditions ( $t(15)=0.719$ ,  $p=0.48$ ) and experimenter rated sleepiness of the child was actually greater following the nap

compared to wake ( $t(18)=4.87$ ,  $p<0.001$ ). More importantly, performance in the nap condition remained superior when recall was again probed the following morning, after nighttime recovery sleep for the wake condition ( $t(22)=2.824$ ,  $p=0.01$ ; Fig. 2). This latter result also suggests the long-term benefits of the nap on memory consolidation.

Greater change in accuracy across the nap trended towards a positive correlation with nap duration ( $r=0.304$ ,  $p=0.058$ ) but was not predicted by age ( $r=-0.037$ ,  $p = 0.84$ ). To avoid ceiling performance, a 9-item grid was used for children <44 months; a 12-item grid was used for older children. To assess whether the number of items encoded (9 v. 12) influenced the change in performance over the nap, we conducted an ANOVA with Condition (nap v. wake) as a within-subjects measure and Items (9 v. 12) as a between subjects factor. Importantly, Condition remained significant ( $F(1,36)=6.256$ ,  $p=.017$ ) while the interaction of Condition x Items was not significant ( $F(1,36)=0.506$ ,  $p=.481$ ).

Nap habituality was also examined with respect to the nap benefit. This measure was derived from caregiver reports of nap frequency. We defined habitually napping children ( $n = 17$ ) as children who napped five days or more per week on average and non-habitually napping children ( $n = 10$ ) were defined as children who napped less than two days per week. The benefit of the nap relative to wake was greatest for children who napped habitually ( $t(16)=2.561$ ,  $p=0.021$ ). Performance of non-habitually napping children did not benefit from an intervening nap ( $t(9)=0.347$ ,  $p=0.736$ ). As seen in Figure 3, this difference was driven by less decay of the memory over wake for the non-habitual compared to the habitually napping children; however, this difference was not significant ( $t(26)=-1.528$ ,  $p=0.139$ ). There was also no difference in the change in

accuracy over the nap for habitually and non-habitually napping children ( $t(25)=0.809$ ,  $p=0.426$ ).

From these results, it may be argued that for children who nap regularly, sleep plays merely a passive role, protecting memories from interference from waking activities (Ellenbogen, Hulbert, Stickgold, Dinges & Thompson-Schill, 2006). This explanation seems unlikely given that in both conditions, 2-3 hrs of wake passed before the approximately 1 hr nap. Thus, decay would be expected in both groups. Rather, in the 4-5 hrs between immediate and delayed recall, there was no change in recall in the nap condition ( $t(38)=0.351$ ,  $p=0.727$ ). As such, we posit that the nap may actively restore the memory following waking interference (Brawn, Nusbaum & Margoliash, 2013). To examine whether memories were actively processed over the nap, we recruited a group of 14 additional children (8 females;  $M=49.83$  months,  $SD=10.17$ , range=33-66 months) who completed the nap condition in the sleep laboratory, with polysomnography recorded during the nap.

Mean length of the laboratory-recorded nap was similar to that of the classroom naps ( $M=73.83$  mins,  $SD=19.92$ ). Naps contained little ( $n=4$ ; 1-10 mins) to no ( $n=10$ ) REM sleep. Rather, naps were largely composed of non-REM stage 2 and slow wave sleep (non-REM stage 3; Table 1). There was a significant negative correlation between sleep spindle density and immediate (baseline) recall performance ( $r=-.665$ ,  $p=0.010$ ), consistent with recent reports that spindle activity has a negative association with IQ (Geiger et al., 2011) and specific aspects of cognitive performance (Chatburn et al., 2013). More importantly, sleep spindle density was positively correlated with the change in memory performance across the nap period (a measure which accounts for variation in

baseline performance;  $r=0.647$ ,  $p=0.012$ ), such that a greater magnitude of sleep benefit was associated with greater density of sleep spindles during the nap (Fig. 4). This relationship cannot be attributed to potential age-related differences in sleep physiology as the relationship between spindle density and age was not significant ( $r=-0.077$ ,  $p=0.792$ ). Other correlations between sleep-dependent performance changes and physiological measure of sleep (e.g., percent time in stage 2 or 3 non-REM) did not survive correction for multiple comparisons ( $\alpha=.01$ ; all  $p$ 's  $> 0.08$ ).

## Discussion

These results show that a mid-day nap in the preschool classroom benefits classroom learning. Following a nap, children recalled 10% more of the spatial locations than when they had been kept awake during the nap opportunity. This effect cannot be attributed to differences in alertness or inattention due to nap deprivation as the nap benefit remained the following day, after overnight sleep, which should equate these factors across the two conditions. Moreover, we find that the negative effects of nap-deprivation on memory consolidation cannot be reversed with overnight sleep.

Importantly, this is the first study of sleep-dependent consolidation in children to examine the physiological basis for performance benefits from sleep. Sleep-dependent changes in recall were specifically associated with sleep spindle density. Such an association would not be expected if the nap merely protected the memory from waking interference. Studies in adults (Fogel, Nader, Cote & Smith, 2007) and, more recently, children (Chatburn et al., 2013) suggest that sleep spindle activity represents a trait-like marker of cognitive abilities. For example, in adults, sleep spindles are positively correlated with IQ (Fogel, Nader, Cote & Smith, 2007) while in children, there is a

consistent negative association between spindle activity and IQ (Geiger et al., 2011) and other cognitive measures (Chatburn et al., 2013). Consistent with this literature, using immediate recall as a proxy for cognitive abilities, we found a negative correlation between spindle density and immediate recall. Notably, in the present study, by using a difference score in which baseline performance is subtracted out, trait-like differences are accounted for and yet the nap benefit remains. Thus, even though spindles are still developing between 2 and 5 years of age (Tanguay, Ornitz, Kaplan & Bozzo, 1975), they can contribute to state-like changes in performance.

Sleep spindles reported here were non-REM stage 2 spindles (few SWS spindles were present) that are likely to be the “fast sleep spindles” referred to in studies of adults. Fast spindles have been associated with activation of the frontal cortex and hippocampus (De Gennaro & Ferrara, 2003; Schabus et al., 2007). Consistent with this pattern of activation, studies in young adults have found an association between sleep spindles with learning and plasticity (Gais, Molle, Helms & Born, 2002; Schabus et al., 2004; Schmidt et al., 2006). Likewise, we posit that in the preschool children, spindles may mark hippocampal-neocortical interactions, a process underlying the stabilization and consolidation of the memory.

Sleep conveyed the greatest benefit on learning for children who regularly nap. Consistent with this result, young adults that napped habitually (one or more nap per week) showed performance improvements on a procedural learning task (a “cup-n-ball” game) following a 20-min nap while those that did not nap habitually (two or fewer naps per month) had performance decrements following the nap (Milner, Fogel & Cote, 2006). Moreover, sleep-dependent improvements in performance were associated with sleep

spindle density only for those who napped habitually. These authors suggest that higher power in the spindles and across other frequency bands (alpha, delta) for those that nap habitually may be indicative of more efficient naps. Likewise, in our study, it is possible that children who napped habitually had more efficient naps resulting in greater consolidation. Our laboratory-based sample was largely habitually napping children prohibiting comparison of sleep physiology for those who nap habitually and non-habitually. However, given that over-nap changes were similar for the habitually and non-habitually napping children, we posit that a more likely explanation is that memories are less susceptible to decay over wake for those children who no longer regularly nap as seen in Figure 3. Such an explanation is consistent with the suggestion that the transition from biphasic to monophasic sleep may coincide with brain maturation (Lam, Mahone, Mason & Scharf, 2011). In the less mature brain, memories in short-term (hippocampal) stores may be more susceptible to interference from additional encoding requiring more frequent, efficient consolidation. With brain maturation, the capacity for memories in short-term memory storage may be increased thus decreasing the need for frequent consolidation and with this, naps may become less efficient.

Collectively, these data suggest that mid-day naps in the preschool classroom support the academic goals of early education. While curriculum demands for preschool classrooms are increasing, the benefit of the sleep on learning warrants preservation of the nap opportunity. Moreover, techniques for enhancing preschool naps should be investigated. Currently, preschools largely lack guidelines around the structure of the nap and nap-promotion tools are under-utilized and unstudied. Finally, it is worth considering whether naps may be a target for assisting children with learning delays. Protecting the

nap opportunity for these children may be critical, and nap promotion may enhance learning from interventions aimed at improving acquisition.

## Methods

Seventy-seven preschool children (50 female;  $M=46.8$  months,  $SD=8.91$ , range=36-67 months) were initially recruited from six preschool classrooms. Children were required to have normal or corrected-to-normal vision, and no present or past diagnosis of disordered sleep. Children were excluded from analysis if they were unable to complete the nap ( $n=10$ ) or wake condition ( $n=1$ ), or failed to complete the task ( $n=4$ ). Additionally, while we attempted to avoid ceiling performance by adjusting the number of items encoded and training to criterion (described below), participants ( $n=22$ ) were removed from the analysis if they had ceiling performance (immediate recall=100%). No participants had floor performance (all immediate recall > 22%).

In the morning (~10 am), children briefly previewed a matrix of squares each with a cartoon image of a common noun (e.g., umbrella, policeman, cat). A 9-item grid was used for children <44 months; a 12-item grid was used for older children. After previewing the items for 30 s, the images were virtually “flipped over” (leaving a matrix of cards, each with an identical pattern; Fig. 1A). Subsequently, an image appeared on the right of the screen and the child was to point to the corresponding hidden image in the matrix. During this encoding phase, the image in the selected location would be revealed, providing the participant with feedback regarding their responses. After all items were presented once, items continued to be presented until 7/9 or 9/12 were responded to accurately. The average number of presentations was 2.2 for the 9-item grid and 2.09 for the 12-item grid. Following learning, memory for the spatial location of the



images was probed in the immediate recall phase (Fig. 1B). During immediate recall, children located each item once and no feedback was given, thus providing an accurate measure of memory without additional learning opportunities.

After immediate recall, children went about their regular classroom routine (outside time, lunch) until the regularly scheduled classroom nap opportunity, approximately 1-3 pm. Enrolled children were either wake- or nap-promoted (within-subject; order counterbalanced; conditions separated by 1-3 weeks). In both conditions, the room was darkened and quiet and children remained on their individual cots/mats as per the typical classroom naptime routine. In the wake condition, children were given quiet activities as needed to encourage wakefulness. In the nap condition, children were encouraged to nap with verbal encouragement and soothing per typical classroom techniques (e.g., back and foot rubbing). Experimenters recorded nap onset and wake time (and any mid-nap wakings) for all enrolled children. After the nap opportunity, delayed recall, identical to immediate recall, was tested (Fig. 1B). At delayed recall, experimenters and children also separately rated child sleepiness on a modified Visual Sleepiness Scale (Maldonado, Bently & Mitchell, 2004). The following morning, memory was probed in the 24-hour recall phase. Due to absences or scheduling conflicts, only 23 children completed this probe for both conditions.

Caregivers completed the Child Sleep Habits Questionnaire (Owens, Spirito & McGuinn, 2000) to assess the child's sleep habits and screen against sleep disorders. Habitually napping children (n=17) were defined as children who napped five days or more per week on average and non-habitually napping children (n=10) were defined as

children who napped less than two days per week according to the Child Sleep Habits Questionnaire.

All procedures were approved by the local Institutional Review Board. As such, parental consent and child assent was obtained before testing commenced.

### Polysomnography

To examine whether sleep-dependent changes in memory were associated with a specific sleep process, we recorded polysomnography during a laboratory-based nap in an additional group of children ( $n=14$ ; 8 females;  $M=49.83$  months,  $SD=10.17$ , range=33-66 months). Enrollment criteria, in addition to those described above, required that the child be likely to nap in the laboratory. Consistent with this, 7 children were reported to nap habitually (5-7 naps per week) and 6 napped often (3-4 naps per week). Only one child did not nap habitually (0-2 naps per week). Following the encoding and immediate recall phases (~1pm), a montage including two EOG, two chin EMG, and five cortical EEG leads (F3, F4, C3, C4; referenced to Cz) was applied. Data were scored for sleep stages according to the revised AASM manual (Iber, Ancoli-Israel, Chesson & Quan, 2007). Sleep spindles were visually detected by a trained physiologist.

### Statistical Analyses

Two-tailed paired-samples t-tests were used to determine differences in immediate, delayed, and 24-hour recall accuracy, as well as the difference score (delayed minus immediate recall), between the nap and wake conditions. Separate analyses were conducted for habitually and non-habitually napping groups. To determine the association between the change in performance across the nap (difference score) and nap

length, spindle density (number of spindles per minute of non-REM stage 2) and other physiological variables, Pearson correlation coefficients were used. The Sidak correction for multiple comparisons was used such that p-values must be below  $\alpha = 0.01$  to be considered statistically significant.

**ACKNOWLEDGEMENTS.** This work was supported in part by NIH R01 HL111695 (R.M.S.C.) and a Commonwealth College Honors Research Grant (K.D.).

Table 2.1. Descriptive variables for polysomnography-recorded naps in 14 children.

Sleep variable	Minutes (SD)	Percent (SD)
Time in bed	96.15 (20.69)	
Total sleep time	73.83 (19.92)	
Sleep latency	14.39 (10.11)	
non-REM stage 1	5.86 (3.18)	7.94 (4.39)
non-REM stage 2	34.04 (17.59)	42.09 (14.90)
non-REM stage 3	32.82 (10.91)	46.19 (17.15)
REM	1.11 (2.81)	1.32 (3.03)

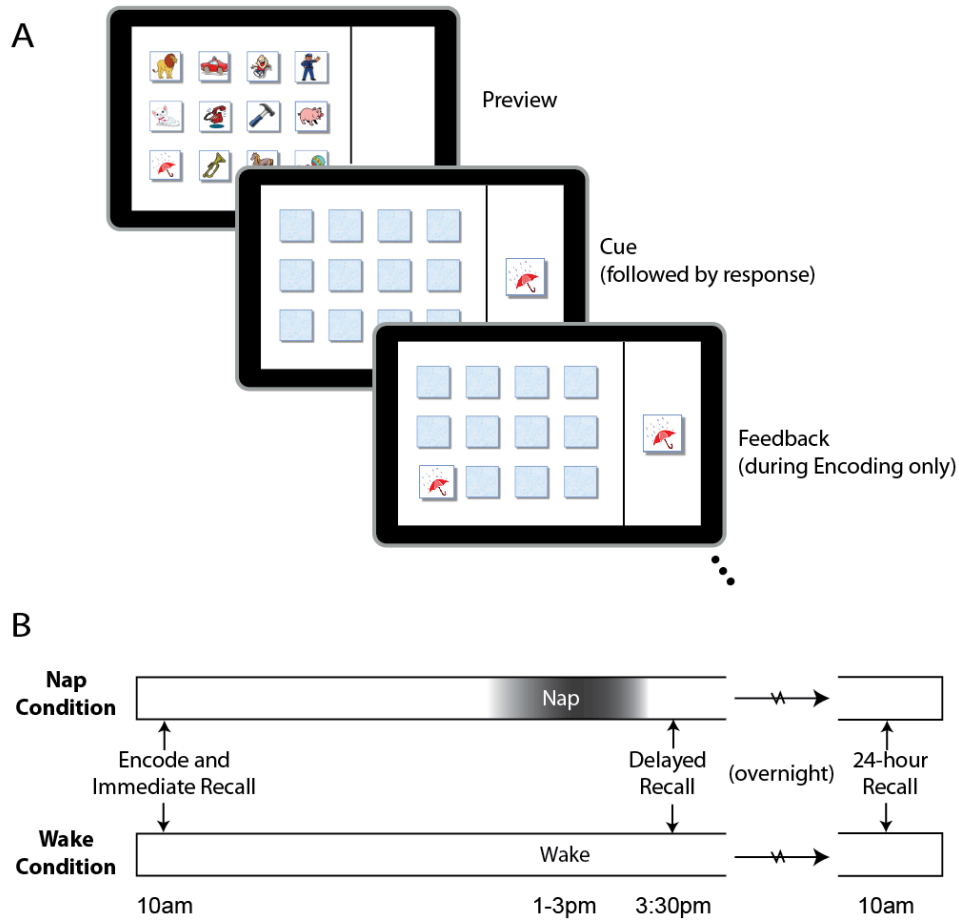


Figure 2.1. Task design and procedures. (A) Preschool children learned the spatial locations of 9- or 12-items on a grid. Following a brief preview, one item was presented on the right side of the screen and children were asked to locate the item by pointing to its position. During the Encoding Phase, feedback was provided. This phase continued until performance reached 75% accuracy. (B) Immediately following the Encoding Phase, Immediate Recall was tested. Children again responded as to the location of each item but feedback was not provided. During the afternoon nap opportunity, children were either nap- or wake-promoted and Delayed Recall was tested shortly afterwards. Recall was tested once again the following morning (24-hour Recall).

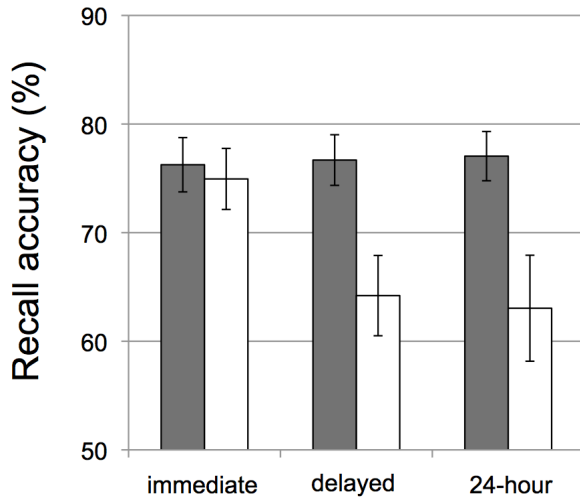


Figure 2.2. Recall accuracy between nap and wake conditions across all phases. Recall accuracy was tested immediately following encoding (Immediate), following the nap opportunity (Delayed) and again the following day (24-hour) across two conditions: A nap-promoted condition (grey bars) and wake-promoted condition (white bars). Error bars represent +/- 1 SE.

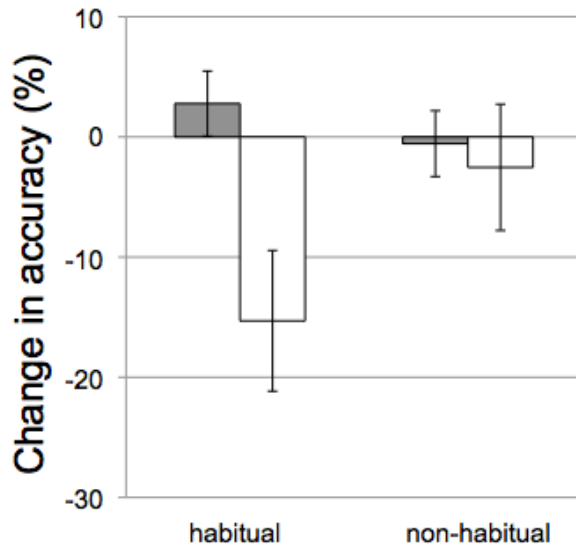


Figure 2.3. Change in recall accuracy across nap habituality groups. Change in recall accuracy (Delayed Recall minus Immediate Recall) across the nap (grey bars) and wake (white bars) intervals for those that took 5-7 naps per week (Habitual) and those that took 0-2 naps per week (Non-habitual). Error bars represent +/- 1 SE.

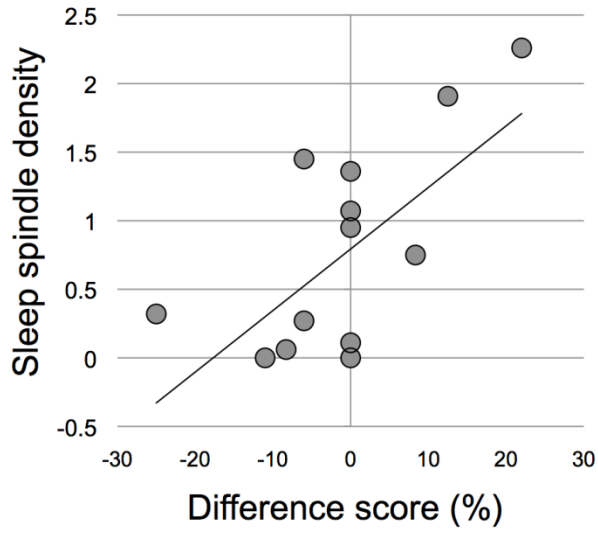


Figure 2.4. Relationship between spindle density and recall accuracy. Sleep spindle density (spindles per minute of non-REM stage 2 sleep) is plotted compared to the change in recall across the nap.



## CHAPTER 3

### THE ROLE OF NAPPING ON EMOTIONAL MEMORY CONSOLIDATION IN PRESCHOOL CHILDREN

Preschool children rapidly develop affective knowledge (Denham, 1986) and become capable of managing their emotions at this age (see review: Cole, Michel & Teti, 1994). Frontal lobe development during the preschool years has been associated with improvement of both emotion regulation (Garon, Bryson & Smith, 2008) and social competence (Fox, et al., 1995). In addition, the improvement in linguistic abilities during preschool also influences the capacity to express and understand emotions (Cutting & Dunn, 1999; de Rosnay & Harris, 2002). With the expansion of the social environment – through peers, teachers, and new siblings – the recognition, comprehension, and control of emotions becomes increasingly important in early childhood.

Greater emotional awareness and understanding in the preschool age range has a number of long-term benefits. For example, increased emotional knowledge in early childhood predicts future positive social relationships (Denham, McKinley, Couchoud & Holt, 1990; Cutting & Dunn, 1999) and socio-emotional development (Leerkes, Paradise, O'Brien & Calkins, 2008). Early emotional understanding is also predictive of long-term academic success (Leerkes et al., 2008). In addition, improved education on affective knowledge, even as early as preschool, is considered to be a mechanism to reduce future bullying and improve the way both victims and potential defenders of those victims respond to bullying (Belacchi & Farina, 2010). Thus, promoting emotional learning and regulation in early childhood may be of critical importance given the devastating and longitudinal effects of bullying in childhood and adolescence (see review: Rigby, 2003).

Indeed, interventions focused on teaching emotional understanding are now common within preschool curricula (Domitrovich, Cortes & Greenberg, 2007; Denham & Burton, 1996). For example, the Preschool PATHS program (*Promoting Alternate Thinking Strategies*; Domitrovich, Cortes & Greenberg, 2007) is a curriculum designed to promote socio-emotional development in preschool children with the goal of improving school readiness. Through it, children are taught to understand, express, and recognize emotions. They are also taught to use pro-social skills (e.g. sharing, taking turns, etc.), and how to problem solve in emotional situations. The PATHS program is successful in improving emotional knowledge and emotional competence, as well as school readiness when compared to control interventions (Domitrovich, et al., 2007; Conduct Problems Prevention Research Group, 2010).

Emotional interventions are one of many programs being implemented in preschool curricula to improve long-term educational and health outcomes, including physical activity (Adamo et al., 2014; Alhassan & Whitt-Glover, in press), nutrition (Hu et al., 2010; Sweitzer et al., 2010), and dental hygiene (Jackson et al., 2005) education. However, these programs often come at the expense of other aspects of the traditional preschool schedule. Specifically, lacking scientific evidence of a function of classroom naps, nap opportunities are at risk of being eliminated. In chapter 2, I provided support for maintaining the nap as part of the daily preschool routine. A daytime nap within the preschool classroom led to significantly improved retention of declarative information learned in the morning when compared to a day without a nap. However, given the importance of emotional processing at this age, there is a need to examine whether napping in preschools is also beneficial to emotional memory consolidation.

The idea that emotions are processed over naps is supported by years of anecdotal evidence from parents and teachers who contend that nap-deprived children are emotionally dysregulated. A recent study in toddlers supports this observation (Berger, Miller, Seifer, Cares & LeBourgeois, 2011). When children were nap-deprived, they rated positive pictures as less positive, and neutral and negative pictures as more negative compared to when they had napped. Nap-deprivation also led to a reduction in positive emotional responses to an enjoyable task (solving a puzzle) and increased negative emotional responses to a task designed to elicit frustration (an unsolvable puzzle) in contrast to the napping condition. In sum, children were better able to manage their emotional responses when they had napped, and demonstrated more negative emotions overall when nap-deprived.

The nap may therefore be necessary to allow children to better process their emotions in the latter half of the day. We posit that this emotion regulation may come about through the consolidation of emotional information attained prior to the nap. If emotional information is consolidated across the nap bout, the resulting liberated cognitive resources could be allocated to new emotional situations. Nap deprivation could therefore prevent the appropriate processing of emotions due to insufficient resources and a greater signal-to-noise ratio. This hypothesis is in line with the synaptic homeostasis hypothesis (Tononi & Cirelli, 2003) that states that while synaptic potentiation and plasticity are required for new learning, neuronal potentiation is energetically expensive and there are limited synaptic resources. As such, a period of sleep is necessary for downscaling and renormalization of synaptic strength in order for further learning to occur efficiently (see review: Tononi & Cirelli, 2014). Given their significantly greater

synaptic density (Huttenlocher, 1979), children may expend synaptic resources at a greater rate than adults. Therefore, an additional daytime bout of synaptic downscaling in the form of a nap may be necessary for children to continue to acquire and process new information successfully throughout the rest of the day (e.g., when faced with new emotional episodes in the afternoon).

Emotional processing (Lara-Cassasco, Nielsen, Solomonova, Levrier & Popova, 2009; Baran, Pace-Schott, Ericson & Spencer, 2012) and emotional memory consolidation (Groch, Wilhelm, Diekelmann & Born, 2013) have been shown to occur over REM sleep in adults. During REM sleep, the amygdala, hippocampus, and cortical areas show a distribution of activation which may support REM's benefit on emotion processing (Maquet, 1996). However, in chapter 2, I demonstrated that the preschool nap contains very little, if any REM sleep. As such, REM is an unlikely candidate for emotional memory consolidation across the preschool nap, should a nap benefit be observed.

Alternatively, naps could benefit emotional memory consolidation through SWS. Synaptic downscaling across sleep is associated with slow wave activity (SWA; Tononi & Cirelli, 2003) during SWS. Slow wave activity is the spectral power in the delta frequency band (0.5-4 Hz). Greater SWA reflects greater synchrony of the activity within the central nervous system during sleep (see review: Greene & Frank, 2010). Slow wave activity has been shown to parallel synaptic density (Feinberg & Campbell, 2009) as well as increases in synaptic potentiation (Huber et al., 2004; Huber et al., 2006). Cellular, molecular, and neuromodulatory mechanisms, as well as changes in gene expression all favor SWS, and specifically SWA, as a time for global synaptic downscaling and

synaptic plasticity in the brain (see reviews: Tononi & Cirelli 2003; Tononi & Cirelli, 2014).

Slow wave sleep and SWA have recently been associated with emotional memory consolidation in adults. In one such example, during encoding of picture locations, negative and neutral images were viewed and paired with a semantically related sound. In a subsequent nap, half of the sounds were played while individuals were in SWS. Faster memory judgments were made for the negative pictures that were cued with the auditory stimuli during SWS (Cairney et al., 2014). In another experiment, overnight SWA was pharmacologically increased following the encoding of an emotional text. Significant memory improvements for the emotional text were observed in the group with increased SWA compared to the control group (Benedict, Scheller, Rose-John, Born & Marshall, 2009).

Studies on the physiological correlates of emotional memory consolidation during development are limited. In older children, between the ages of 9-12 yrs, consolidation of emotional images was positively associated with the spectral power of the slow oscillation (>1 Hz) during SWS (Prehn-Kristensen et al., 2013). Slow oscillations reflect global synchrony of neuronal activity within the brain, which organizes sleep rhythms such as slow waves (Massimini, Huber, Ferrarelli, Hill & Tononi, 2004).

The present study examined whether preschool-aged children consolidate emotional memories over a daytime nap. Sleep architecture of the nap and of the subsequent nocturnal sleep bouts was monitored in order to determine the physiological mechanisms that could influence behavioral changes across sleep. As napping has been shown to affect emotional reactivity in children (Berger et al., 2011), it was predicted that

the nap would lead to improved emotional memory consolidation compared to a similar period of wake. In addition, it was predicted that SWS and SWA would be associated with the improvement of memory performance across subsequent sleep periods.

## Methods

### Participants

Sixty-four preschool-aged children (43 female;  $M=51.55$  months,  $SD=7.23$ , range=34-64.3 months) participated in this study. Children were required to have normal or corrected to normal vision and have no diagnosis of sleep (present or past) or neurological disorders, as determined through parent report.

### Task

The task was modified from a short-term emotional memory task used by Kinzler and Shutts (2007) in preschool children. The task had four phases: an encoding phase and three recall phases (Figure 1). During encoding, participants were shown images of neutral expression faces on a computer screen. Faces were taken from the Radboud Faces Database (Langner, Dotsch, Bijlstra, Wigboldus, Hawk & van Knippenberg, 2010) and the Max Planck Institute's FACES collection (Ebner, Riediger & Lindenberger, 2010). During the presentation of each image, a statement was read aloud describing the individual as either "mean" or "nice" (Figure 1; Appendix A). To control for the subject matter of the statements, each mean statement had an equivalent counter nice statement (e.g. Mean: "Mary stole everyone's cookies and no one got any." Nice: "Joel brought in cookies, and everyone got some."). An equal number of male and female face stimuli as well as mean and nice statements were presented at encoding during each session. To prevent ceiling effects, the task difficulty was adapted based on the age of the child.

Older children (> 3.5 years) saw 18 faces for 6 seconds each, whereas the younger children (< 3.5 years) saw 12 faces for 8 seconds each during the encoding phase. Children were instructed to look at the faces and listen to the statements, but were not required to make any responses during encoding. The experimenter monitored that children attended to each stimulus as the statement was read.

During each of the three recall phases (immediate recall, delayed recall, and 24-hour recall) children were given a two-item forced choice task. Children were asked to select the familiar (encoded) face when paired with a novel distractor face, matched for gender. To prevent additional learning, only a subset of the encoded faces was shown during each recall phase. Both the gender and the emotional valence of the stimuli were balanced across each recall phase. Children who encoded 18 faces were given 6 trials at each of the three recall phases, and children who encoded 12 faces were given 4 trials. The first recall phase (immediate recall) provided a baseline measure of memory for the emotional stimuli, whereas the subsequent recall phases (delayed and 24-hour recall) were used to assess the change in memory performance over time.

#### Measures/Questionnaires

Children were asked to rate their level of sleepiness using the visual sleepiness scale (VSS). This measure is reliable (compared to Karolinska Sleepiness Scale in adults,  $\alpha=.72$ , Maldonado, Bentley & Mitchell, 2004) and validated for use in children over 6 yrs (Kollins et al., 2011). As an additional control, experimenters also rated each child's level of sleepiness using the same scale. In addition to the VSS, a visual analogue mood scale (VMS; Folstein & Luria, 1973) was also used for a subjective rating of mood.

This scale has been validated for use in children (Cella & Perry, 1986). As with the VSS, the VMS was used by both the child and the experimenter to indicate the child's mood.

Primary caregivers completed the Child Sleep Habits Questionnaire (CSHQ) to assess each child's sleep habits and sleep health. This assessment is reliable ( $\alpha=.88$ ) and validated for detecting disordered sleep in preschool-aged children (night wakings, parasomnias; Goodlin-Jones, Sitnick, Tang, Liu & Anders, 2008; Owens, Spirito & McGuinn, 2000). This questionnaire was also used to determine the child's typical napping patterns.

Primary caregivers were additionally asked to complete the Child Behavior Checklist/1½-5 (CBCL/1½-5), which was used as an assessment of childhood behavior (Achenbach & Edenbrock, 1983). The CBCL/1½-5 is a widely-used, validated and reliable assessment (test-retest,  $\alpha=.90$ ; Achenbach & Rescorla, 2000). The CBCL/1½-5 measures children's tendencies towards specific behavioral problems, as well as more generalized internalizing (over control of emotions) or externalizing (under control of emotions) behavioral issues.

### Procedures

Each child participated in two experimental sessions (within-subjects design), separated by at least one week. In one session, children were nap-promoted, and in the other session, children were wake-promoted (Figure 2). The order of the nap and wake conditions was counterbalanced across participants. Children were either tested within the preschool classroom ( $n = 44$ ) or in the sleep lab ( $n=20$ ) when sleep physiology was monitored.



## Classroom Study

Children were recruited from local preschools following the approval of the school's director and teachers. Written parental consent was obtained for each child prior to the start of the study, and child assent was obtained before the task was administered. All procedures took place in the preschool classroom. Testing procedures were designed to limit the interruption of the typical preschool routine and to prevent disrupting the children in the classroom who were not consented to be a part of the study.

Encoding typically occurred between 10:00 and 11:00 am. The first recall phase, immediate recall, was conducted immediately after encoding. Following encoding and immediate recall, children went about their typical classroom routine (e.g., outside playtime, lunch).

Nap and wake promotion occurred during the classroom nap opportunity (typically from 1:00 – 3:00 pm). During wake promotion, children were required to remain on their cot/mat in the darkened classroom, but were kept awake with quiet activities such as looking through books and playing with puzzles. During nap promotion, back rubbing and soothing were used to encourage children to sleep.

After the classroom nap opportunity, the second recall phase took place (delayed recall). Children were then asked to rate their level of sleepiness on the VSS. Experimenters also rate the child's level of sleepiness at this time. Following this recall phase, experimenters left the classroom, and children continued with their typical schedule. The following morning, experimenters returned to the classroom for the final phase of recall (24-hour recall), after which both children and experimenters rated the child's sleepiness with the VSS. This concluded the session. Approximately one week

later, children participated in the alternate condition, with all procedures identical to the first week.

### Laboratory Study

Children were recruited for the laboratory study from local area preschools, through community flyers, and through the UMass Developmental Psychology recruitment database. Procedures were identical to the classroom design with a few exceptions. First, all children in the laboratory study were given the 18-item image set in order to have more items in the recall phases to compare with sleep physiology. Second, to better facilitate parent schedules and reduce time in-lab, encoding and immediate recall took place between 11:30 am and 12:00 pm, approximately one hour prior to the nap/wake promotion. Children in the laboratory study were also asked to rate their level of sleepiness using the VSS at the immediate recall phase in addition to the delayed and 24-hour recall phases. Unlike the classroom study, children and experimenters rated the child's emotionality using the VMS at all recall phases. Additionally, primary caregivers were asked to complete the CBCL/1½-5 as well as the CSHQ, whereas in the classroom study they were only asked to complete the CSHQ.

Most importantly, children in the lab were equipped with PSG (described below) during sleep intervals (Figure 2). In the nap condition, after PSG application, children were provided with a two hour nap opportunity in which napping was promoted as in the classroom study. PSG was also applied following immediate recall in the wake condition for the majority of participants ( $n = 18$ ). During this time, children were provided with non-emotional and non-arousing quiet activities (e. g., coloring, puzzles) for two hours in

place of the nap opportunity. As in the classrooms, nap and wake promotion occurred at the same time of day (~1-3 pm) across conditions and participants.

Following the two-hour nap/wake opportunity, delayed recall was assessed in the same manner as the classroom study. After delayed recall, children and parents left the lab to complete the rest of their daily routine. For both the nap and wake sessions, parents brought their child back to the lab in the evening, approximately one hour prior to the child's typical bedtime. The PSG montage was applied again, and the child slept overnight in the lab. The 24-hour recall occurred approximately 30 minutes after awakening, following removal of the PSG montage and morning routines (e.g., teeth-brushing, changing clothes) in order to prevent sleep inertia from biasing recall measures.

#### Polysomnography

For the first two subjects, a 14-electrode montage of electroencephalography (EEG), electromyography (EMG), and electrooculography (EOG), was used. This included six cortical EEG leads (F3, F4, Cz, C3, C4, O1, and O2), and two EOG electrodes, all referenced to electrodes placed on the mastoids (A1 and A2). In addition, a ground electrode was affixed to the forehead, and two EMG electrodes were placed on the chin and referenced to each other. For the remaining 18 children, a 32-electrode EEG cap (BrainVision Easy Cap), customized with 2 EMG and 2 EOG electrodes, was used for physiological recordings. In addition to the electrodes used in the smaller montage, the EEG cap included 18 additional cortical EEG leads (Fz, F7, F8, FCz, FC1, FC2, FC5, FC6, CP1, CP2, CP5, CP6, Pz, P3, P4, P7, P8, and POz) also referenced to electrodes placed on the mastoids (A1 and A2).

## Frontal Alpha Asymmetry

The EEG Cap was also applied during wake-promotion for the last 18 participants. EEG recordings during this restful waking period were used to assess frontal alpha power asymmetry, which is an indicator of trait-like affective response to emotional stimuli (see review: Davidson, 1992) in adults (Tomarken, Davidson & Henriques, 1990; Wheeler, Davidson & Tomarken, 1993) as well children (Harmon-Jones & Allen, 1997; McManis, Kagan, Snidman & Woodward, 2002). A right-bias in alpha power in the anterior regions of the brain is associated with depression and negative affect, whereas a left-bias is associated with positive affect. To determine whether trait-like differences in responsiveness to emotional stimuli would affect the participant's ability to consolidate emotional memories, a frontal alpha asymmetry measure was obtained. In line with previous work, EEG recordings were taken while children sat quietly. EEG was recorded for four consecutive one-minute epochs (Harmon-Jones & Allen, 1997; McManis, Kagan, Snidman & Woodward, 2002). In the first and third epoch, the lights were turned off in the room, and in the second and fourth epoch, the lights were turned on in the room. Following these recordings, children continued to wear the EEG cap across the delay period in order to mimic the time wearing the cap in the nap condition.

## Data Analysis

### Behavioral Data

Three types of difference scores were calculated for this experiment. To assess changes in memory over naps and equivalent intervals of wake, a difference score was calculated in which baseline memory performance was subtracted from delayed recall

(Delayed Difference Score = delayed recall – immediate recall). The Delayed Difference score was also used to compare how nap sleep physiology affected memory performance change across this delay. The Overnight Difference Score (24-hour recall – delayed recall), examined how memory changed across the overnight sleep period alone, regardless of how memory changed across the daytime. The Overnight Difference Score was used to compare how nocturnal sleep physiology affected memory performance change across the overnight delay period. A third type of difference score, the 24-hour Difference Score was calculated to measure the change across the entire 24-hour period compared to baseline performance (24-Hour Difference Score = 24-hour recall – immediate recall). This measure incorporates the processing of information across both the nap and the nocturnal sleep bout in the nap condition, thereby allowing for a potential interaction between the two. Comparatively, in the wake condition, this measures the change across nocturnal sleep after an entire period of daytime waking.

To examine differences in emotional memory consolidation, 2x2 repeated measures ANOVAs were run comparing the calculated difference scores with condition (Nap vs. Wake) and emotion (Mean vs. Nice) as within-subjects factors. Nap habituality, as determined by parent report on the CSHQ, was also used to determine whether sleep benefits are greater for habitually napping children than for non-habitually napping children. A habitually napping child was defined as a child who naps 5-7 times per week on average, and a non-habitually napping child was defined as a child who naps, at most, twice a week.

## Polysomnography Data

PSG recordings were coded for sleep stages using the American Academy of Sleep Medicine (AASM) criteria (Iber, Ancoli-Israel, Chesson & Quan, 2007), developed for healthy young adults and suitable for preschool age children (e.g. Meltzer, Walsh, Traylor & Westin, 2011; Chapter 2). Additionally, spectral analyses of PSG were run using BrainVision Analyzer 2 software (Ver 2.4; Brain Products), with specific interest in delta and sigma power. Data were segmented into the sleep stage of interest (SWS for delta power, and both SWS and nREM2 for sigma power). Data were then analyzed using the periodogram method for spectral analysis (Welch, 1967). Staging data were segmented into 4-second epochs for artifact rejection on individual channels, and a fast-Fourier transform (FFT) was applied using a Hanning window with 10% overlap and utilizing covariance. This method is standard for EEG analysis of sleep data (Achermann, 2009). A spectral power range of 0.5-4 Hz was used for delta power analyses, and a range of 11-16 Hz was used for sigma power. Spectral analyses are reported in power density ( $\mu\text{V}^2/\text{Hz}$ ).

Paired-samples t-tests were used to assess differences in overnight sleep physiology between the nap and wake conditions. Additionally, measures of sleep physiology were correlated with changes in memory performance across the sleep condition using Pearson's correlations. Lastly, linear regressions were used to determine whether specific sleep measures within the nap predicted the differences in memory performance across nocturnal sleep following the nap and wake conditions.

### Frontal Alpha Asymmetry Data

BrainVision Analyzer 2 software was also used to examine frontal alpha asymmetry. EEG data for the lights on and lights off periods were processed separately. The same pre-processing and artifact detection steps used for sleep data were used for the EEG data. Following FFT, spectral power density ( $\mu\text{V}^2/\text{Hz}$ ) in the alpha frequency range (8-13 Hz) was obtained from the frontal electrodes (specifically F3 and F4). A log transformation was applied, and an alpha asymmetry score was calculated between the right and left hemispheres ( $\text{Log R} - \text{Log L}$ ). A composite alpha asymmetry score of lights on and lights off periods were then correlated (Pearson's correlations) with memory performance.

### Questionnaire Data

The CSHQ was used to ensure that no children in the study had a history or diagnosis of a sleep disorder. In addition it was used to identify the napping status (habitual or non-habitual) of the child. The CBCL/1½-5 was used to quantify behavioral profiles of the children in the laboratory study. Of particular interest was the Internalizing Behavior score, which includes emotional reactivity, anxious/depressive behaviors, somatic complaints, and social withdrawal symptoms. This measure, as well as the Externalizing Behavior score, was used to assess whether differences in trait behavior lead to differences in emotional memory consolidation. Pearson's correlations were used to compare the Internalizing Behavior score and the Externalizing Behavior score with performance at immediate recall, as well as difference scores across delay periods.

## Results

### Descriptives

Of the 64 children, 1 was unable to be wake promoted, 3 were unable to be nap promoted, and 3 were absent on one of the main testing days. All of these cases were from testing in the classroom study. Therefore, useable data from 57 children were collected with 37 of those children tested in the classroom, and 20 children tested in the laboratory. To ensure that children were getting sufficient sleep during the nap, only children whose nap total sleep time was above the mean ( $M = 71.92$  min), or within one standard deviation ( $SD = 22.99$  min) below the mean were used ( $n = 49$ ; 30 female;  $M = 51.55$  months,  $SD = 7.16$ , range = 34-64.3 months). This criterion was considered important as, in the nap physiological data collected in Chapter 2, children completed their main bout of SWS on average  $45.5 \pm 7.19$  min after sleep onset. Children napped for a similar period of time in the lab ( $M = 70.88$  minutes,  $SD = 24.37$ ) as they did in the classroom study. In addition, 2 children were unable to complete the 24-hour recall phase due to absences from school on the follow-up days. Their data were still included in the delayed recall analyses.

### Memory Performance

#### Immediate Recall

Considering participants across both the classroom and laboratory study, at immediate recall, baseline differences in accuracy were observed between the nap ( $M = 63.65\%$ ,  $SD = 24.58$ ) and wake conditions ( $M = 72.36\%$ ,  $SD = 19.53$ ;  $t(48) = -2.254$ ,  $p = 0.029$ ). These differences cannot be explained by order effects as order was counterbalanced across participants and there was no significant main effect of session



order on performance across conditions ( $F(1, 47) = 0.505, p = 0.481$ ). Sleepiness and mood were not collected at immediate recall for the classroom study. However, for the laboratory study, there were no differences between conditions in children's self report of sleepiness or mood following immediate recall ( $p$ 's  $> 0.05$ ). The experimenter ratings were also not different for the child's mood, but experimenters did rate children as significantly more sleepy after immediate recall for the nap condition compared to the wake condition ( $t(18) = 2.348, p = 0.031$ ).

#### Over Nap/Wake Performance Changes

There were no significant main effects of condition ( $F(1,47) = 0.678, p = 0.414$ ; Figure 3), or emotion ( $F(1, 47) = 0.285, p = 0.596$ ; Figure 4) on the Delayed Recall Difference Score. There was also no significant interaction between condition and emotion ( $F(1, 47) = 0.733, p = 0.396$ ; Figure 4). Performance was not affected by age, as there were no significant associations between age and the Delayed Difference Score for either the nap ( $r = 0.036, p = 0.806$ ) or the wake condition ( $r = -0.167, p = 0.251$ ).

#### Overnight Performance Changes

Across the overnight sleep period alone (Overnight Difference Score), there were no significant main effects of either condition ( $F(1,42) = 1.084, p = 0.304$ ) or emotion ( $F(1,42) = 0.910, p = 0.345$ ). There was also no significant interaction between condition or emotion ( $F(1,42) = 1.063, p = 0.3085$ ) despite trajectories in the predicted directions (Figure 5).

The 24-hour Difference Score was analyzed to determine how performance changed across the entire testing period, from initial baseline performance to performance following overnight sleep. There was a significant main effect of condition on the 24-

hour Difference Score ( $F(1, 43) = 4.523, p = 0.039$ ; Figure 3). The nap condition led to protected memory for the encoded items whereas the wake condition resulted in significant forgetting (one-sample t-test;  $t(45) = -2.819, p = 0.007$ ). There was no significant main effect of emotion ( $F(1, 43) = 0.234, p = 0.631$ ), nor was there a significant interaction between condition and emotion ( $F(1, 43) = 0.286, p = 0.595$ ) on the 24-hour Difference Score (Figure 4). Age of the child was not significantly associated with their 24-hour Difference Score for either the nap ( $r = 0.164, p = 0.272$ ) or the wake condition ( $r = 0.227, p = 0.130$ ).

Nap habituality was also examined with respect to the role of napping on the 24-hour Difference Score. In the full sample, there were 15 children meeting the criterion of ‘habitually napping’ and 10 children classified as ‘non-habitually napping’. The significant main effect of condition was observed only for those children who nap habitually ( $F(1, 13) = 7.581, p = 0.016$ ) and not in the non-habitually napping children ( $F(1, 8) = 0.943, p = 0.360$ ; Figure 6).

### Sleep Physiology

In the lab, the nap was primarily comprised of SWS, as well as nREM2 sleep (Table 1). Only 6 of the 20 children (30%) reached REM sleep in their nap. When REM occurred, it comprised only 8.11% (SD = 4.38) of the nap bout.

The night following the nap, children took longer to fall asleep ( $t(19) = 2.612, p = 0.017$ ), had reduced total sleep time ( $t(15) = -3.173, p = 0.006$ ), and reduced sleep efficiency ( $t(15) = -2.35, p = 0.033$ ) compared to the night following the wake condition. On the nap condition night, a significantly larger proportion of the night was spent in nREM1 sleep ( $t(15) = 3.37, p = 0.004$ ) than on the wake condition night. Conversely, a

significantly smaller proportion of the night was spent in SWS on the night following a nap than on the night of the wake condition ( $t(15) = -3.62, p = 0.003$ ). There were no differences in percent of the night spent in either nREM2 sleep ( $t(15) = 1.03, p = 0.322$ ) or REM sleep ( $t(15) = 0.501, p = 0.624$ ) across conditions. There were also no significant differences in spectral frequencies in the delta or sigma bands overnight across conditions, in either the frontal or central cortical leads.

### Relationship Between Sleep Physiology and Memory Performance

Across all participants, total nap sleep time was not correlated with the Delayed Difference Score in the nap condition ( $r = 0.019, p = 0.906$ ). Nap physiology was examined in association with over-nap changes in memory consolidation for the participants in the laboratory study. As most children ( $n = 14$ ) did not reach REM sleep in the nap, performance change across the nap bout was not assessed with respect to this sleep stage. Instead, memory performance changes were compared with time spent in SWS and SWA across the nap. While the Delayed Difference Score was not significantly correlated with the percentage of the nap spent in SWS ( $r = -0.347, p = 0.146$ ), there was a significant negative relationship with SWA ( $r = -0.601, p = 0.008$ ; Figure 7).

Therefore, greater delta power in the nap was related to poorer memory performance directly following the nap period. This result was not likely due to greater sleep inertia following a deeper sleep in the nap, as neither the child's nor the experimenter's ratings of sleepiness (VSS) or mood (VMS) were correlated with either SWA in the nap or the Delayed Difference Score following the nap (all  $p$ 's  $>0.05$ ).

The relationship between nocturnal sleep physiology and change in memory performance was examined. The Overnight Difference Score was not significantly

correlated with the percentage of night spent in REM sleep for either the nap condition ( $r = -0.350$ ,  $p = 0.131$ ) or the wake condition ( $r = 0.055$ ,  $p = 0.840$ ). There was, however, a significant positive correlation between the Overnight Difference Score and the percentage of the night spent in SWS for the nap condition ( $r = 0.488$ ,  $p = 0.029$ ; Figure 8). This relationship was not observed for the wake condition ( $r = 0.140$ ,  $p = 0.606$ ; Figure 8). Moreover, memory for the mean faces in particular was significantly correlated with SWS percentage for the nap condition ( $r = 0.556$ ,  $p = 0.011$ ; Figure 9). No significant relationship was observed across the wake condition, nor was a significant relationship seen for nice faces in either the nap or the wake condition (all  $p$ 's  $> 0.05$ ). Performance changes in memory were not associated with any other sleep stage.

Given that overnight SWS was reduced in the nap condition compared to the wake condition, it is interesting that positive associations between SWS and memory were observed only in the nap condition. As such, time spent in SWS alone cannot fully explain the differences in memory performance between the two conditions. To better understand what factors in the nap condition resulted in memory performance improvements across the night, an Overnight Nap Benefit Score was calculated. This score represents how much performance improved overnight in the nap condition compared to the wake condition ((Nap 24-hour recall – Nap Delayed recall) – (Wake 24-hour recall – Wake Delayed recall)). Linear regressions were used to identify factors that predict the Overnight Nap Benefit Score. The difference in SWS percentage between the two overnight periods did not predict the difference in performance ( $b = -0.112$ ,  $t(17) = -0.479$ ,  $p = 0.638$ ). Similarly, percentage of SWS in the nap did not predict the difference in performance across the two nights ( $b = 0.026$ ,  $t(17) = 0.107$ ,  $p = 0.916$ ). Importantly,

SWA in the nap did significantly predict the performance changes across the overnight sleep periods ( $b = 0.475$ ,  $t(17) = 2.159$ ,  $p = 0.046$ ; Figure 10). Nap SWA, in turn, showed a near-significant correlation with the percentage of the nocturnal sleep bout spent in SWS ( $r = 0.422$ ,  $p = 0.066$ ), which may explain this relationship. As previously stated, SWA in the nap bout was significantly negatively correlated ( $r = -0.601$ ,  $p = 0.008$ ; Figure 7) with the change in performance across the nap period. Overall these results suggest that greater SWA during the nap leads initially to reduced memory performance, but then facilitates the improvement of memory performance across the nocturnal sleep bout.

#### Trait-Like Individual Factors

Additional assessments were used to determine whether trait-like differences in emotional reactivity or behavior would affect emotional memory consolidation on this task. First, there were no associations between measures of interest on the CBCL/1½-5 and scores at immediate recall or any of the difference score measures for either the nap or wake condition. Specifically, memory performance was not associated with Internalizing or Externalizing Behaviors, and was not associated with the specific subscale of emotional reactivity (all  $p$ 's  $> 0.05$ ). Secondly, there were no significant correlations between memory performance and measures of frontal alpha asymmetry (all  $p$ 's  $> 0.05$ ). This indicates that the results of this study were not biased by trait-like differences in emotional reactivity or typical behaviors.

#### Discussion

This chapter examined whether emotional information is consolidated across a nap and subsequent overnight sleep in preschool-aged children. An immediate benefit of

a nap on emotional memory consolidation was not observed in this population. However, a nap led to significantly improved memory for emotional stimuli when recall was probed again the next day. Slow wave activity during the nap as well as time spent in SWS during the nocturnal sleep bout was associated with the delayed memory improvements in the nap condition.

#### Behavioral Changes in Memory Performance Across Sleep

Individually, the nap and the overnight sleep bouts were not sufficient to induce changes in memory. Across the nap and wake period (Delayed Difference Score) and across nocturnal sleep (Overnight Difference Score), performance was equivalent between conditions. Only when the changes across the entire 24-hour period were considered (24-Hour Difference Score) was a significant benefit of napping was observed. In the nap condition, the 24-Hour Difference Score includes processing across both episodes of sleep (nap and nocturnal sleep) in the memory outcome. In the wake condition, the 24-Hour Difference Score reflects changes across the afternoon of waking as well as across the subsequent nocturnal sleep bout. The significant differences between conditions in the 24-Hour Difference Score suggest an interplay between the nap and subsequent overnight sleep in the consolidation of emotional memories.

Comparatively, a day of continuous waking led to significant forgetting, which could not be reversed with overnight sleep. The results support that the nap led to stabilization and protection of new memories from forgetting even though these effects were not apparent directly following the nap/wake manipulation.

## The Role of Emotional Valence on Behavioral Changes in Memory Across Sleep

Surprisingly, there were no significant main effects of emotion observed in any of the measures in this study. Previous research indicates that preschool-aged children preferentially remember mean faces compared to nice faces across a short delay (Kinzler & Shutts, 2007). As such, mean faces in this study were also predicted to be preferentially remembered over nice faces. However, while mean faces were remembered better overall in the Kinzler and Shutts study, at an individual level fewer than half of their participants (17 out of 38) showed preferential memory for mean faces. Five children showed a memory benefit for the nice faces, and 16 of the children showed equivalent memory for mean and nice faces. In the current study, the percentage of children with either a mean (36.2%) or nice (37.9%) bias was comparable at immediate recall. The remaining children (25.9%) showed no bias for either the mean or the nice faces. Thus, preschool-aged children are quite variable with respect to a negative or positive bias in memory.

Importantly, few studies have examined positive and neutral stimuli together in the same experiment – rather, most compare emotional stimuli to neutral stimuli (e.g. Prehn-Kristensen et al., 2009; Baran et al., 2012). In adults, memory for positive and negative stimuli has been compared across sleep and sleep deprivation conditions. Under conditions of normal sleep, no significant differences were observed for memory accuracy of positive and negative pictures (Sterpenich et al., 2007) nor for recognition of happy and angry faces (van der Helm, Gujar & Walker, 2010). Following sleep deprivation, positive pictures are forgotten compared to the negative stimuli, but both happy and angry faces show equivalent performance deficits following sleep deprivation.

Therefore, in adults, emotional valence does not consistently bias the consolidation of emotional memories across sleep.

In children, positive and negative stimuli are also consolidated similarly across sleep. Memory for angry and happy faces was not significantly different following sleep, although both were remembered better than neutral expression faces (Prehn-Kristensen, Mozlow, Wiesner & Baving, unpublished data). Interestingly, slow oscillation power during SWS was only associated with memory for the angry faces. These results closely mimic those observed in the current experiment where both mean and nice stimuli were remembered equivalently over sleep but only the mean faces were associated with nocturnal SWS. Overall, the results of the current experiment are in line with other studies of emotional memory consolidation in which positive and negative stimuli are combined.

The emotional response to this task may have been different if alternative stimuli were used. It has been suggested theoretically that children would show a bias towards the processing of own-age faces over those of adults (see review: Rhodes & Anastasi, 2012). However, recent evidence opposes this theoretical framework. Children show greater ability to recognize adult faces compared to peer-aged faces (Macchi Cassia, 2011; Macchi Cassia, Pisacane & Gava, 2012), and activation in the face processing neural network has been shown to be greater for adult compared to child faces (Marusak, Carre & Thomason, 2013). This bias towards adult face processing was demonstrated across positive and negative emotional expression faces, and was also shown in neutral expression adult faces. Thus, the use of adult faces in the current study, as opposed to own-age faces, is appropriate and likely beneficial for stimuli recognition.



## Physiological Correlates of Behavioral Changes Across Sleep

Emotional memory consolidation in adults has been primarily attributed to REM sleep (e.g., Groch, Wilhelm, Diekelmann & Born, 2013). Yet, most of the children in this study (30%) did not reach this stage in their nap. Thus, REM sleep is unlikely to play a role in nap-dependent emotional memory consolidation in preschool-aged children. Although REM sleep was achieved in the nocturnal sleep bout, time spent in this sleep stage was not associated with memory performance in either the nap or the wake condition. The consolidation of emotional memory therefore may rely on different physiological processes than adults.

Given the relationship between SWA and synaptic downscaling (see review: Huber & Born, 2014) as well as synaptic plasticity (Campbell & Feinberg, 2009), SWA was also considered a potential candidate for emotional memory consolidation across a nap. Slow wave activity (Benedict, Scheller, Rose-John, Born & Marshall, 2009) and SWS (Cairney et al., 2014) have been associated with improved performance on emotional tasks in adults. In addition, SWA has been suggested to be involved in cortical development to optimize performance gains (Kurth et al., 2012). However, in this study, SWA in the nap was negatively correlated with performance changes across the nap bout – greater SWA was associated with worse performance upon waking. This result was counter to our predictions.

An alternative way in which the physiology of the nap could influence delayed recall of memories is indirectly, through the manipulation of nocturnal sleep physiology. Changes in nocturnal sleep may be the result of alleviation of homeostatic pressure across the nap. Previous work in adults suggests that differences in homeostatic sleep pressure

are represented by differences in nocturnal SWA. Typically, SWA increases following periods of sleep deprivation or prolonged waking, and SWA decreases across periods of sleep (see review: Greene & Frank, 2010). In the present study, overnight SWA was not significantly different between conditions suggesting that homeostatic sleep pressure at night was similar. While this may seem counter to what has been reported previously, it is important to note that nocturnal sleep physiology following a day with and without a nap has never been examined within this developmental population. Further, only 5 of the 20 participants with sleep physiology were reported to nap habitually and no child napped 7 days a week on average. Seven of the children were categorized as non-habitually napping, and the remaining 8 children napped 3-4 times per week on average. Therefore, a day without a nap may not be equivalent to “sleep deprivation” for the majority of the children and is well within the range of normal for our sample.

Despite similarities in SWA, differences in overnight sleep physiology did exist between the nap and wake conditions. Napping during the day actually led to comparatively poorer overnight sleep quality than the wake condition. Following a nap, children had reduced total overnight sleep time (TST), reduced sleep efficiency, and spent a greater percentage of the night in the lightest sleep stage (nREM1) compared to when they had been kept awake during the day. Therefore, these differences do not seem to be a plausible explanation for the benefit of napping observed in this study.

In addition, children spent less of the night in SWS when they had napped during the day than when they had been kept awake. Interestingly, memory improvements across the nocturnal sleep period were positively associated with time spent in SWS, but only in the nap condition. If nocturnal SWS alone were sufficient for emotional memory

consolidation, the wake condition should have outperformed the nap condition. Further, the difference in time spent in nocturnal SWS did not predict differences in offline memory performance change between conditions. This supports the notion that changes in nocturnal sleep physiology are not adequate to explain the differences in performance between conditions and again implicates an interaction between napping and overnight sleep in the consolidation of emotional memory.

### The Role of Napping and Overnight Sleep on Emotional Memory Consolidation

Both the behavioral and physiological data support that processing over the nap was necessary for the protection of memory across the entire 24-hour period. To better understand this interaction, the relationship between nap physiology and performance changes across overnight sleep was assessed. Greater SWA in the nap predicted the extent to which memory was benefitted by nocturnal sleep in the nap condition compared to the wake condition (Overnight Nap Benefit Score). As such, nap SWA may be important for the processing of emotional memories and lead to more efficient consolidation across subsequent sleep periods.

Interestingly, nap SWA was associated with the improvement in memory across subsequent overnight sleep despite being negatively correlated with memory performance directly following the nap. While this is a unique finding within the human literature, a very similar effect has been demonstrated in juvenile zebra finch song learning (Derégnaucourt, Mitra, Fehér, Pytte & Tchernichovski, 2005). In this study, vocal replication of a model song deteriorated when a period of sleep directly followed initial learning; however, the magnitude of deterioration following sleep was positively correlated with eventual similarity to the model song. Therefore, poorer performance

following sleep was associated with better performance in the long-term. This indicates an active but delayed role of sleep in learning.

In response to this effect, Deregnacourt and colleagues (2005) ran additional control studies to better understand the deterioration of song performance following sleep. They found that neither sleep inertia nor circadian effects could explain this result. Even a pharmacologically induced daytime sleep bout using melatonin was sufficient to induce performance deficits following sleep. The authors concluded that changes across sleep could represent synaptic (Bottjer & Johnson, 1997) or cellular (Nordeen & Nordeen, 1988) remodeling possibly through neuronal replay during sleep (Dave & Margoliash, 2000). They posed that greater remodeling during the initial sleep period results in the greater deterioration of performance in the subsequent waking period but is critical to promote learning. Consolidation would continue in subsequent periods of processing. Thus, maximal improvements in long-term performance would require greater remodeling both during the initial sleep bout and during additional processing time later. Deregnacourt and colleagues suggest that this delayed benefit of sleep and the oscillation between initial deterioration and later improvement in performance are likely strongest in juvenile populations. This may be why the finding has not been reported in other studies of sleep and memory performance.

The relationship between emotional memory consolidation and nap physiology in preschool-aged children mimics the results of the Deregnacourt study in juvenile zebra finches. While napping did not lead to overall deterioration of emotional memory in preschool-aged children, the association between nap SWA and memory did alternate between deterioration and subsequent improvement. This is intriguing as the effects in

this study were also observed in a juvenile population. Given the similarities between these results and those of the Deregnacourt study, SWA during the nap may similarly reflect synaptic remodeling and plasticity.

This potential connection between SWA and synaptic remodeling is supported in the literature as SWA has been shown to be tightly linked with cortical plasticity (See review: Tononi & Cirelli, 2003). The synchrony of neuronal activation during SWA produces regular and periodic neurotransmitter release as well as changes in intracellular calcium concentrations that facilitate plasticity within neuronal populations (see review: Bennington & Frank, 2003). Studies have shown that SWA increases or decreases respectively in cortical regions that are more or less active during previous waking in rats (Vyazovskiy et al., 2000) as well as in humans (Huber et al., 2004; Huber et al., 2006). The relationship between SWA and cortical plasticity was also examined by Huber and colleagues (2008) using transcranial magnetic stimulation (TMS). In this study, TMS was used to mimic synaptic potentiation and synaptic depression within the cortex. In subsequent sleep, SWA was locally increased in the potentiation-like condition, and locally reduced in the depression-like condition. These studies strongly indicate that SWA is directly associated with cortical plasticity.

Napping in preschool-aged children may therefore begin the process of synaptic remodeling of the learned emotional memories. But, those memories require additional processing time over successive sleep periods to promote observable differences in memory performance. Greater plasticity over the nap, as indicated by greater SWA, may allow nocturnal SWS to be more effective at consolidating and preserving the memories learned earlier in the day.

In contrast, with the lack of processing and remodeling directly following learning, information is forgotten across the 24-hour period in the wake condition. Newly formed memories may be more susceptible to daytime waking interference (Diekelmann, Büchel, Born & Rasch, 2011) without plastic changes across the nap. Additionally, SWS during the nocturnal sleep period in the wake condition was not sufficient to induce memory benefits. Sleep deprivation has been shown to reduce neuronal excitability and long-term potentiation of synaptic strength in rats (McDermott, et al., 2003). While wake-promotion of the children in this study was not necessarily sleep deprivation as previously discussed, prolonged periods of waking following learning may reduce the neuronal excitability of the involved synaptic circuits. This could therefore diminish the likelihood of localized SWA acting to induce plastic changes to stabilize and transfer information into long-term storage during nocturnal sleep.

### Conclusions

The results of this study support that napping is important for emotional memory consolidation in preschool-aged children. Despite the delayed nature of the memory benefits observed, the nap was critical to elicit memory improvements, and nocturnal sleep alone was not sufficient to consolidate emotional information learned during the day. Given that emotional information is prevalent and significant in the daily environment of a preschool-aged child, napping may be critical for long-term emotional aptitude. Within the preschool classroom, a large proportion of the curriculum is dedicated to teaching children about recognizing, understanding and dealing with emotional information. As such, napping should greatly facilitate the educational experience. This study further indicates that despite a possible reduction in overnight

sleep quality, there are significant cognitive and emotional benefits of a daytime nap in preschool-aged children. This research supports the persistent promotion of napping in preschool children as a means to enhance learning and memory outcomes, especially within the realm of emotional education.

## CHAPTER 4

### GENERAL DISCUSSION

The aim of this dissertation was to examine the role of napping on sleep-dependent memory consolidation (SDC) in preschool children. Children were taught either a declarative or an emotional task in the morning, and memory was probed following a nap and following an equivalent period of wake. Memory was also examined 24-hours later in the morning after overnight sleep. Overall, memory for both declarative and emotional information was shown to benefit from the nap. In both experiments, wake-promotion during the day led to an approximate 10% reduction in memory accuracy the following morning, whereas napping resulted in an overall protection of information.

#### Differences in Nap Dependent Consolidation Across Memory Systems

Interestingly, the mechanism through which this nap benefit occurred appears to be unique for the declarative and the emotional tasks. As presented in Chapter 2, declarative information was protected in the nap condition compared to significant forgetting in the wake condition. This difference between conditions was observed directly following the nap. Spindle density was associated with better performance across the nap period, and the difference between conditions was sustained the following morning. These results suggest that the nap actively consolidated declarative information and led to long-term stability of the memories. Comparatively, as presented in Chapter 3, emotional information did not show an immediate benefit of napping. Rather, napping in conjunction with overnight sleep resulted in a delayed benefit on memory performance. Slow wave activity during the nap was related to this benefit. Therefore, although the



effect was delayed, the nap was still necessary for the protection of newly formed emotional memories.

To explain the dissimilarities in the trajectories of nap-dependent memory consolidation, the most discernable distinction between the two experiments is that the tasks are designed to probe different forms of memory. These two types of memory rely on particular, albeit overlapping, brain networks. Declarative memory is predominantly hippocampus-dependent (Rasch, Büchel, Gais & Born, 2007), whereas emotional memory processing involves not only the hippocampus but also additional brain regions such as the amygdala (Whalen et al., 1998; Hamann, Ely, Hoffman & Kilts, 2002), the anterior cingulate gyrus, and the prefrontal cortex (see review: Phillips, Drevets, Rauch & Lane, 2003). Furthermore, the emotional task used here also included a social component. Thus, encoding of the emotional task was likely associated with a broader brain activation pattern compared to encoding of the visuospatial task.

Given the different brain networks involved in encoding the two memory types, it is perhaps not surprising that distinctive physiological measures would be related to consolidation of these memories. Sleep spindles were associated with the consolidation of visuospatial memories across the nap. There is a strong temporal relationship between sleep spindles and sharp-wave ripples (Siapas & Wilson, 1998; Sirota, Csicsvari, Buhl & Buzsáki, 2003; Clemens et al., 2007), which are high frequency (100 – 300 Hz) oscillations generated in area CA3 of the hippocampus (Wilson & McNaughton, 1994), suggesting a relationship between hippocampocortical and thalamocortical systems (see reviews: Marshall & Born, 2007; Diekelmann & Born, 2010). One theory as to the function of this synchronicity is that sharp-wave ripples bias spindle activity towards

particular cellular networks connected to those that had been potentiated within the hippocampus during encoding (Werk, Harbour & Chapman, 2005). Spindles cause a substantial influx of calcium into cortical pyramidal cells, predisposing neocortical synapses to plasticity and long-term potentiation (Rosanova & Ulrich, 2005; Steriade, 2006; see reviews Marshall & Born, 2007; Diekelmann & Born, 2010). This could therefore be the mechanism through which targeted memories are transferred from hippocampal to neocortical stores. Given that the visuospatial task of Chapter 2 was primarily hippocampal in nature, the association between improvement across the nap and spindle events is in line with this theory.

Conversely, SWA during the nap as well as SWS overnight were associated with the delayed benefit for emotional memories. Slow wave sleep is characterized by global organization of activity within the brain. Greater SWA during SWS reflects both greater synchronization of neuronal firing and a larger number of contributing neuronal networks. The oscillating release of neurotransmitters and the rhythmic changes of intracellular calcium concentrations that are reflected in SWA possibly mediate plastic changes within the brain on a more comprehensive scale (see review: Greene & Frank, 2010). Slow wave sleep has therefore been suggested to benefit performance on tasks which bind features from different memories and different memory systems (see review: Diekelmann & Born, 2010). As the emotional task includes declarative, emotional, and social information and relies on a broadly distributed neural system, SWS and SWA are ideal candidates for the consolidation of this task.

The visuospatial and emotional tasks also likely differ with respect to the amount of previously consolidated relevant information. Sleep reorganizes and incorporates new

information into pre-existing networks of long-term memories (see review: Diekelmann & Born, 2010). Children may have had previous experience with a memory task similar to the visuospatial task (e.g., the concentration game). However, the mean and nice statements of the emotional memory task were designed to mimic possible scenarios, both negative (e.g., children not sharing, toys being broken) and positive (e.g., prosocial behavior, partaking in fun activities), that children would be exposed to on a daily basis. It is therefore reasonable to assume that children had a larger quantity of previously stored memories that aligned with the emotional task in comparison to the visuospatial task. Thus, during consolidation, the integration of the newly formed emotional memories would have been more global and involve more cortical networks than that of the newly formed visuospatial memories. The need for such widespread integration could further explain the role of SWS and SWA in the consolidation of these emotional memories. Also, the additional processing time needed for the emotional information to be consolidated (delayed as opposed to immediate benefits of napping) could reflect the greater diversity of networks into which the new information needed to be incorporated.

In sum, differences were observed between the sleep mechanisms involved in the nap-dependent consolidation of declarative and emotional memories. These distinctions can be accounted for by differences in the scope of the brain networks involved, as well as by the array of relevant memory networks into which the new information is integrated.

#### The Role of Napping Within the Preschool Classroom

Napping led to improved recall of both declarative and emotional information within the preschool classroom environment. This work supports the continued practice

of nap promotion as part of the typical preschool schedule. Napping can be used to benefit academic success of children by improving the retention of information gained in the preschool classroom. Despite reductions in nocturnal sleep efficiency following daytime napping, there were long-term benefits of the preschool nap in both declarative and emotional memory realms.

In the state of Massachusetts, there have recently been changes to the requirements for resting and nap promotion within preschool classrooms. Prior to 2010, requirements included a minimum of a 45-min rest period for children when in care for longer than 4 hours per day. However, in January of 2010, the Massachusetts Regulations for Family, Group and School Age Child Care Programs and the standards for licensure stated the more vague requirement that states all preschools must allot a sleep, rest, or quiet activity period that is “appropriate to the needs of the children.” A consequence of this ambiguity could be the loss of the preschool nap opportunity should parents, teachers, or directors determine that napping is no longer a sufficient need of the child. Already, some schools within Western Massachusetts currently provide alternative options to a rest period for children. For example, in some preschools parents can opt to have their child play in a separate room or take additional lessons such as French language or swimming classes while other children are napping. A lack of understanding about the academic benefits of the nap may result in parents making a well intentioned but unhealthy decision for their child with regards to nap promotion. While this policy requirement stands, it is necessary that preschools accurately determine the appropriate sleep need for the children within their classrooms.

Importantly, the frequency with which napping occurred was relevant to both the declarative and the emotional tasks. In both experiments, the significant benefit of napping was observed primarily in the habitually napping children. Non-habitually napping children did not show significant forgetting of information across the waking delay as did the habitually napping children. Previous work by Lam and colleagues (2011) implicates brain maturation in the transition to monophasic sleep patterns in preschool children. The current data also supports this relationship. With increasing brain maturation, newly learned information could be maintained within short-term hippocampal stores for longer periods of time. Therefore, the benefits of continued nap promotion in preschool classrooms will decrease as individual children mature. Notably, within the preschool population, nap habituality and brain maturation do not always align with the age of the child. As such, age is not a reliable indicator of the appropriate time for children to transition to monophasic sleep, nor should it be used to determine appropriate sleep need.

#### Future Directions

Future research into the role of napping on memory, emotion, and behavior will be important for policy change with respect to nap promotion in preschool classrooms. A future direction of this research would be to use this data to inform parents, teachers, and directors about the benefits of napping within the preschool-age range. In addition, these studies may be critical to inform policy makers about the need for greater regulations surrounding nap promotion within the preschool classroom. Meanwhile, further research to help identify an objective measure of sleep need is necessary to ensure that children within preschool classrooms are being provided with the appropriate nap opportunities.

Napping and nap frequency may have other effects that should be considered which are outside the scope of this dissertation. Chapter 3 demonstrated that napping led to a comparatively reduced overnight sleep quality compared to a day of wake. However, this relationship is likely mediated by the habituality with which naps occur for each child. This could not be analyzed within our sample due to insufficient power. Also, it is unclear whether napping resulted in poorer sleep quality, or if wake promotion led to above-average sleep quality as a result of sleep debt acquired across daytime waking. Napping is not consistent within this population, with most children napping between 2-5 days per week. As such, it is difficult to establish a normal baseline of nocturnal sleep physiology with which to compare between nap and wake conditions.

Along similar lines, it has yet to be examined whether the consistency of napping has long-term effects on sleep patterns. For example, following a day without a nap a child may sleep longer and more deeply during the subsequent night, thus decreasing the probability of napping the following day. Comparatively, longer and more frequent daytime naps may result in a reduced nocturnal sleep quality thereby increasing the likelihood of a subsequent nap. As long as the pattern remains consistent, children are likely to reach an equilibrium between sleep distribution and sleep need. However, most children of this age group are not consistent in their napping schedules. The inconsistency of napping could lead to an imbalance between sleeping and sleep need, which could have greater implications for cognitive and emotional outcomes. Therefore, although this dissertation demonstrated significant benefits of napping on memory consolidation across a 24-hour period, it may be that irregular napping has greater negative consequences than discontinuing napping altogether.

Future research is necessary to determine when to transition to a completely monophasic sleep pattern in order to optimize overall cognitive and emotional gains. In addition, rather than completely prohibiting napping, future studies should identify ways in which sleep patterns can be stabilized in preschool-aged children while including daytime sleep. For example, greater emphasis on nap promotion or a stricter and more consistent napping schedule may be sufficient to habituate the nap. There is a high prevalence of sleep-onset difficulties in preschool children (Petit, Touchette, Tremblay, Boivin & Montplaisir, 2007), and therefore children may need additional encouragement to sleep during the day as they do at night. Reducing nap time or changing the time of day in which the nap occurs may also be useful techniques to ensure that the homeostatic drive towards sleep is strong at the child's ideal bedtime.

Finally, this dissertation examined only the role of napping on memory consolidation. Napping may affect a number of other factors that could influence learning during the preschool age. For example, if synaptic downscaling occurs across the nap, children should have greater synaptic resources to improve learning efficiency in the latter half of the day. Napping could also improve attention, decision making, or executive functions in preschool-aged children. As such, further research is necessary to fully comprehend the role of napping in the cognitive development of the child, as well as in the effectiveness of learning within the preschool classroom.

Table 3.1. Sleep characteristics from polysomnography

	Nap Mean (SD)	Nap Overnight Mean (SD)	Wake Overnight Mean (SD)	<i>p</i> *
TST (min)	70.88 (24.37)	522.68 (71.34)	549.51 (74.76)	.006
Sleep Latency (min)	15.03 (10.04)	44.70 (31.35)	26.73 (21.00)	.017
WASO (min)	11.35 (16.32)	37.08 (40.14)	36.39 (28.22)	.550
Sleep Efficiency (%)	73.06 (17.78)	86.54 (7.94)	90.03 (6.41)	.033
nREM1(%)	9.39 (4.49)	9.39 (2.51)	8.42 (2.19)	.004
nREM2 (%)	36.85 (13.52)	53.22 (4.78)	50.31 (9.05)	.322
SWS (%)	51.20 (15.36)	19.73 (4.74)	23.89 (7.00)	.003
REM (%)	2.56 (4.51)	17.67 (3.60)	17.41 (3.96)	.624
SWA C3 ( $\mu\text{V}^2/\text{Hz}$ )	312.07 (67.52)	244.42 (76.96)	243.39 (65.20)	.970
SWS Sigma C3 ( $\mu\text{V}^2/\text{Hz}$ )	3.02 (2.09)	1.84 (1.13)	1.87 (0.99)	.933
nREM2 Sigma C3 ( $\mu\text{V}^2/\text{Hz}$ )	4.09 (3.21)	2.29 (1.61)	2.22 (1.21)	.354
Nap spindle density	0.52 (0.75)	n/a	n/a	n/a

TST, total sleep time; WASO, wake after sleep onset; nREM, non-rapid eye movement sleep, SWS, slow wave sleep; REM, rapid eye movement, SWA, slow wave activity.

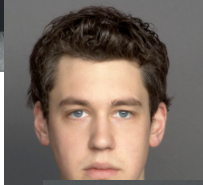
\* *p* values correspond to paired samples t-tests comparing nap and wake overnight bouts.



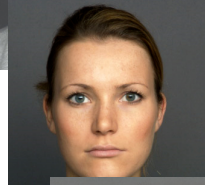
### Encoding:



*"This is my friend Faye. Faye is always mean. Today she stole everyone's cookies and no one got any."*



*"This is my friend Rufus. Rufus is always mean. Today he spilled milk all over the table and didn't help clean it up."*



*"This is my friend Stella. Stella is always nice. Today she shared a box of new crayons with everyone."*



*"This is my friend Jude. Jude is always nice. Today he brought in cookies and everyone got some."*

### Immediate Recall:



*"Which one of these is our friend Faye?"*

### Delayed Recall:



*"Which one of these is our friend Stella?"*

### 24-Hour Recall:



*"Which one of these is our friend Rufus?"*

Figure 3.1. The emotional memory task.

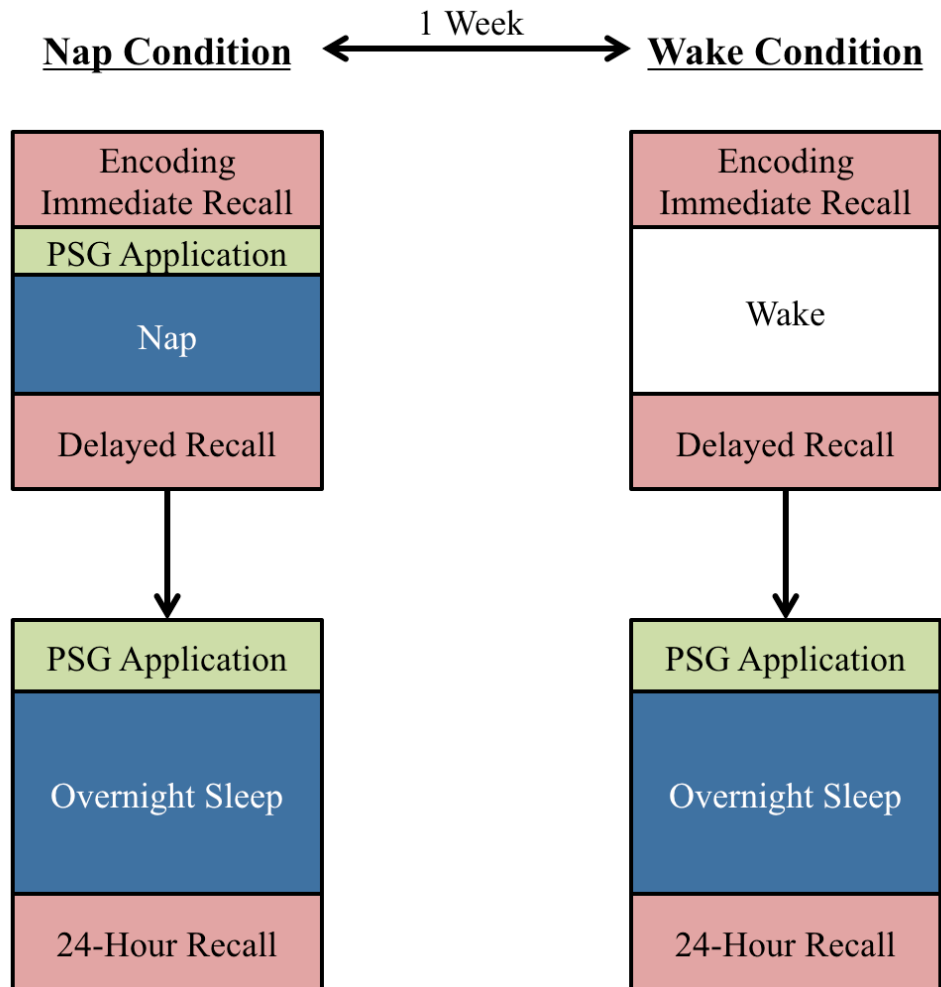


Figure 3.2. An overview of the study procedures. All children participated in the encoding and recall phases. Only children in the laboratory study (n=20) participated in the physiological recordings of sleep with polysomnography (PSG).

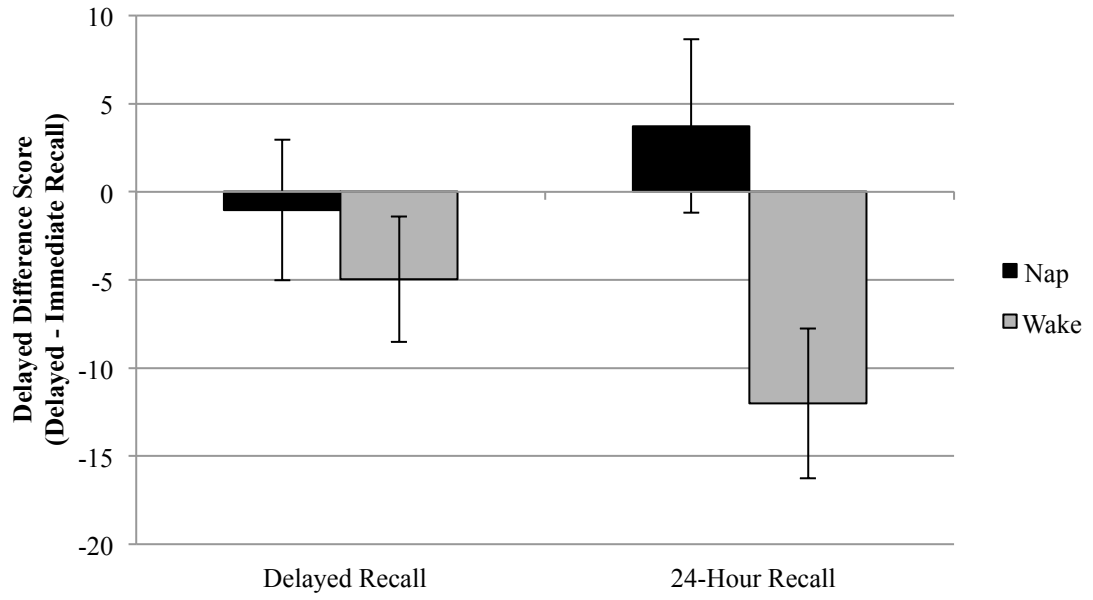


Figure 3.3. The change in emotional memory accuracy across the delay periods, controlling for baseline.

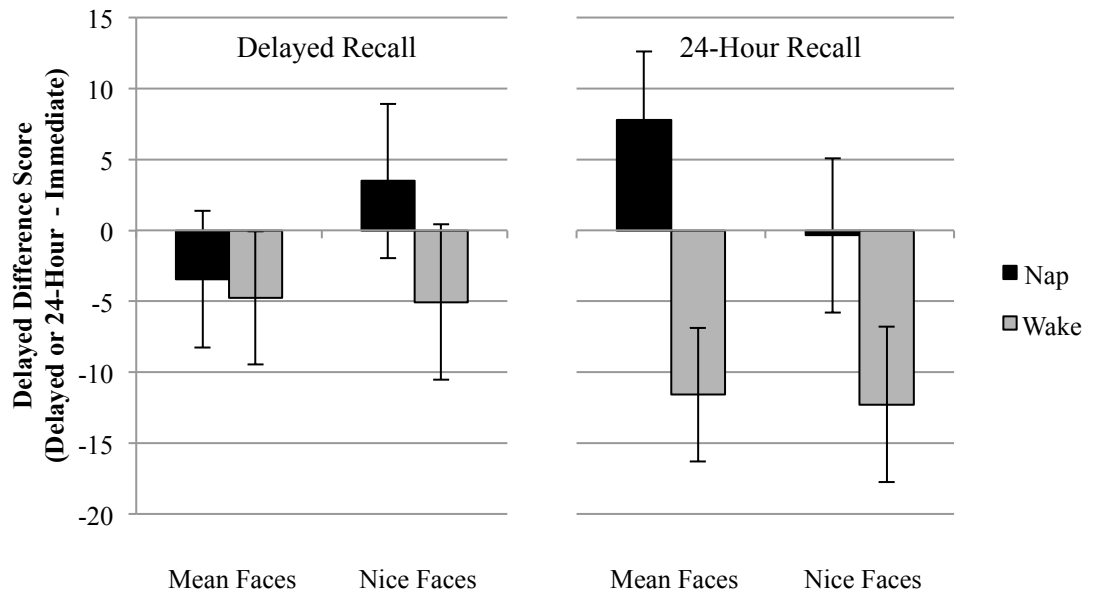


Figure 3.4. The change in emotional memory accuracy across the delay periods, controlling for baseline, and separated by valence of stimuli.

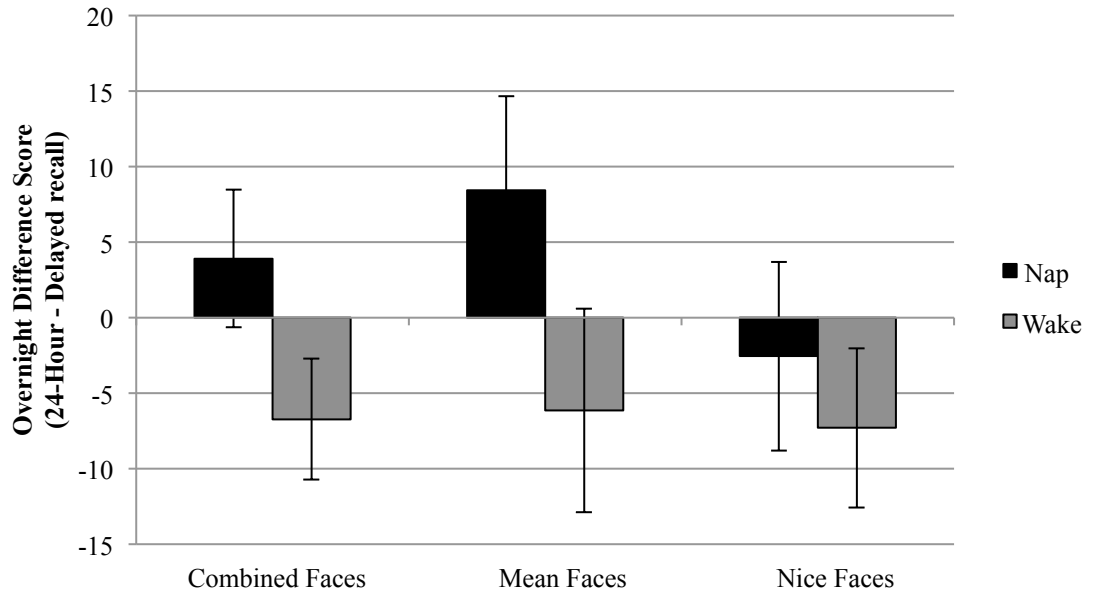


Figure 3.5. The change in emotional memory accuracy across the overnight period. This includes overall performance change and separation by valence category.

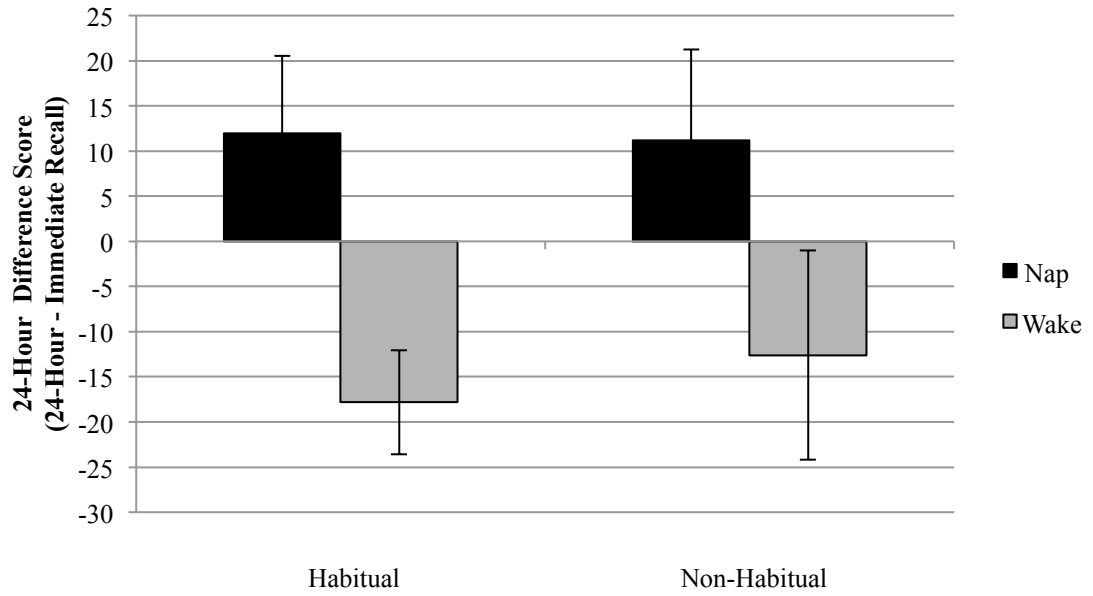


Figure 3.6. Change in recall accuracy across nap habituality groups. The change in emotional memory accuracy across the 24-hour delay period, controlling for baseline and separating performance for habitually napping children (napping 5-7 days per week) and non-habitually napping children (napping 0-2 days per week).

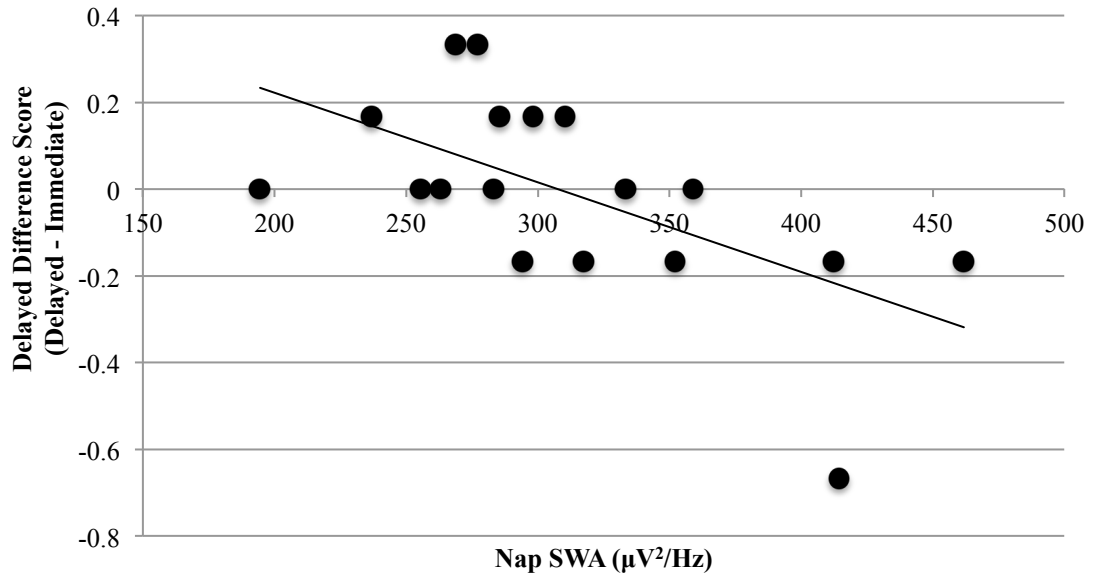


Figure 3.7. The inverse correlation between SWA in the nap and the performance change across the initial delay period ( $p = 0.008$ ).

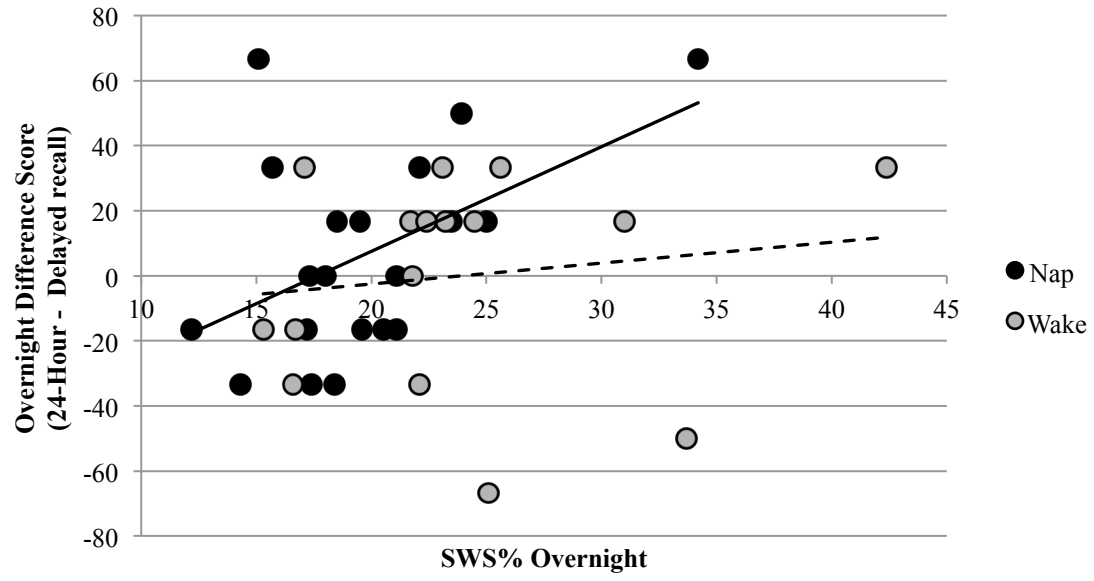


Figure 3.8. The relationship between SWS and overnight memory performance changes. The solid line represents this relationship in the nap condition ( $p = 0.029$ ), whereas the dashed line represents the wake condition ( $p = 0.606$ ).



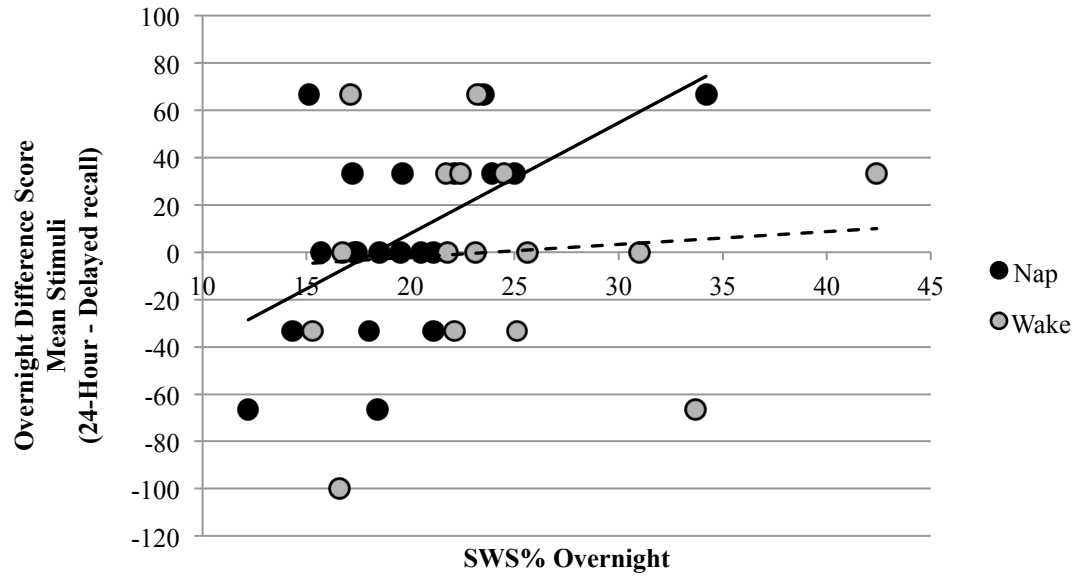


Figure 3.9. The relationship between SWS and overnight memory performance changes for mean stimuli. The solid line represents this relationship in the nap condition ( $p = 0.011$ ), whereas the dashed line represents the wake condition ( $p = 0.760$ ).

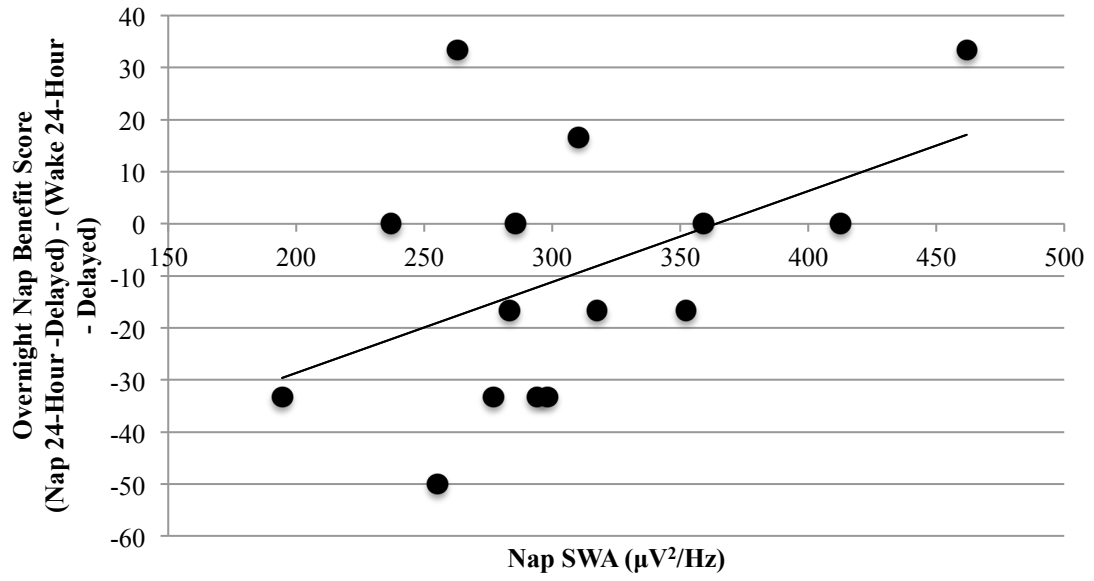


Figure 3.10. The predictive relationship between SWA in the nap and the overall benefit of nocturnal sleep in the nap condition compared to the wake condition ( $p = 0.046$ ).

## APPENDIX

### EMOTIONAL MEMORY STATEMENTS

Mean and nice statements used during the encoding of the emotional memory consolidation task.

List 1 (18 item encode list):

1. Brielle is always mean. Today she broke all of the crayons so that no one could color with them
2. Joel is always nice. Today he rubbed our backs and helped us fall asleep at nap time
3. Hugo is always mean. Today he spilled milk all over the table and didn't help clean it up
4. Jordan is always nice. Today he brought in a book to share with the class
5. Anita is always mean. Today she stole everyone's cookies, and no one got any
6. Gavin is always mean. Today he wrinkled-up the picture the class made
7. Henry is always nice. Today he brought a ball for us all to play with
8. Gwenith is always nice. Today she pushed us on the swings outside
9. Winnie is always nice. Today she shared a box of new crayons with everyone
10. Gus is always mean. Today, he hid the ball so that no one could play with it
11. Faye is always nice. Today she helped us pick flowers outside in the garden
12. Lulu is always mean. Today she was really loud and no one could rest during nap time
13. Stella is always nice. Today she made a pretty painting to hang in the classroom
14. Leena is always nice. Today she helped us pour milk into our cups at lunch time
15. Steven is always mean. Today he wouldn't let us use the swings outside
16. Phillip is always mean. Today, he put the flowers we picked in the garbage
17. Liza is always mean. Today she ripped the book that we were going to read in class
18. Alfred is always nice. Today he brought in cookies and everyone got some

List 2 (18 item encode list):

1. Bette is always mean. Today she wouldn't let us pick the book we wanted to read before nap time
2. Ethan is always nice. Today he told us how pretty our painting was
3. Cedric is always mean. Today he interrupted us while we sang a song for the class
4. Harvey is always nice. Today he helped us clean up the mess we made at lunchtime
5. Rosemary is always mean. Today she wouldn't let go of the butterfly we caught
6. Vincent is always mean. Today he took all the clay away, and no one got to use it
7. Griffin is always nice. Today he brought in a toy to share with the class
8. Celeste is always nice. Today she read us a book to help us fall asleep for nap time

9. Naomi is always nice. Today she brought in fruit for a fruit salad, and everyone got some
10. Asher is always mean. Today he wouldn't let us play outside today
11. Pearl is always nice. Today she sang a really fun song with us
12. Felicity is always mean. Today he wouldn't share the toy he brought in for the class
13. Melinda is always nice. Today she helped us look for butterflies
14. Fiona is always nice. Today she helped us make a town out of clay
15. Leo is always mean. Today he made a mess at lunchtime and didn't clean it up
16. Sheldon is always mean. Today he stole everyone's fruit, and no one got any
17. Camille is always mean. Today she threw our painting away
18. Frederick is always nice. Today he taught us a new game to play outside

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