ABSTRACT

Title of Thesis:	IS NAP STATUS RELATED TO MEMORY, SLEEP PHYSIOLOGY, AND THE HIPPOCAMPUS IN EARLY CHILDHOOD? Tamara Lynn Allard, Master of Science, 2020
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Research suggests there may be links between developmental changes in sleep (e.g., transition out of a nap), memory, and brain (specifically, hippocampus). The purpose of this investigation was to explore differences in sleep physiology, visuospatial memory, and hippocampal volume based on nap status. Participants were 3 to 5-year-old children (n=51) who were habitual nappers (napping >5 days/week), semi-habitual nappers (3–4 days/week), or non-nappers (<2 days/week). Participants completed a memory task before and after a wake and nap session. Polysomnography (PSG) and hippocampal volumes were also assessed. Findings demonstrated that, regardless of nap status, children performed better on a memory task following a nap. PSG revealed that habitual nappers spent marginally more time in nREM2 sleep and less time in SWS compared to semi-habitual nappers. Finally, non-nappers demonstrated a larger hippocampus than the other groups. These findings support the suggestion that developmental differences in these domains are related during childhood.

IS NAP STATUS RELATED TO MEMORY, SLEEP PHYSIOLOGY, AND THE HIPPOCAMPUS IN EARLY CHILDHOOD?

by

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Research indicates that there are marked improvements in episodic memory during early childhood (Riggins, 2014). During early childhood, children also transition from biphasic sleep, consisting of an afternoon nap and an overnight sleep bought, to monophasic sleep (Blair et al., 2012; Galland et al., 2012; Iglowstein et al., 2003). These developments may be connected. Specifically, during sleep, memories are believed to undergo consolidation that leaves them less vulnerable to interference and forgetting. The purpose of this investigation is to assess differences in memory and the brain between children who have and have not undergone this transition.

Neural Mechanisms of Sleep and Memory

In adults, research suggest that sleep plays an important role in declarative memory consolidation, with little evidence to the contrary. Furthermore, research suggest that not only does sleep protect memories, it actively enhances them (Diekelmann & Born, 2010; Rasch & Born, 2013). This enhancement of memory during sleep is thought to reflect transfer of memories from the hippocampus to the cortex due to sleep microstructure. During nREM (non-Rapid Eye Movement) sleep, the hippocampus generates sharp wave ripples believed to represent memory reactivation (Bendor & Wilson, 2012). These ripples have been functionally correlated with short, high-frequency burst of EEG waves called sleep spindles that originate in the thalamus and propagate to the cortex during nREM2 sleep and slow wave sleep (SWS; e.g. Helm et al., 2011; Stickgold & Walker, 2009; Rasch & Born, 2013). Research that utilizes polysomnography (PSG) has demonstrated that sleep

spindles, the percentage of time spent in nREM2 sleep, and the percentage of time spent in SWS are all associated with a memory benefit across overnight sleep and across an afternoon nap (Kurdziel et al., 2018; Kurdziel et al., 2013; Lokhandwala & Spencer, 2020). Together these findings suggest that sleep plays a critical role in memory consolidation and that sleep spindles support offloading of memories from the hippocampus to the cortex.

Development of Sleep and Memory

In early childhood (i.e., ages 3 - 5 years), children spend progressively less time asleep across a 24-hour period (Roffwarg, Muzio, & Dement, 1966). Research suggest that this shift is driven by the transition out of their afternoon nap (Blair et al., 2012; Galland et al., 2012; Iglowstein et al., 2003). Specifically, from infancy through early childhood, children take progressively fewer naps and the duration of naps decreases (Kurth et al., 2016; Ohayon et al., 2004; Weissbluth, 1995). For example, 93% of 3-year-old children nap 6 or more days per week, whereas only 27% of 5-year-old children nap 4 days per week (citation(s) needed – even if included in previous sentence or move from previous sentence to here). Furthermore, children 6 months old nap for an average of 3.5 hours per day, while children aged 6 years nap an average of 1.5 hours per day (Weissbluth, 1995).

Additionally, during early childhood, children demonstrate dramatic improvements in episodic memory performance (Bauer et al., 2007; Riggins, 2014). For example, previous work demonstrates that from age 5 to 7 years, memory performance increases on a lab task where children are asked to remember the source

of new information (Riggins, 2014). Similar findings have been demonstrated with autobiographical memory in children age 3 to 7 (Bauer et al., 2007).

During this same developmental period, research has demonstrated relations between sleep and memory performance across an afternoon nap. This same work also suggests that there are marked differences in memory performance between children who nap habitually (i.e. 5 or more days per week), and those who do not nap habitually (i.e. 2 or less days per week). Although both habitual nappers and non-nappers benefit from afternoon sleep (Kurdziel et al., 2018; Kurdziel et al., 2013; Lokhandwala & Spencer, 2020), habitual nappers' memory is significantly more impaired when they are kept awake (Kurdziel et al., 2013; Kurdziel et al., 2018). Moreover, memories that are lost across a wake session are not recovered during overnight sleep, suggesting that the mid-day nap is critical to memory consolidation during early childhood (Kurdziel et al., 2013; Lokhandwala & Spencer, 2020).

Research has demonstrated that memory change across a nap (i.e., memory after the nap – memory before the nap) is positively related to sleep microstructure and sleep architecture during early childhood. Specifically, sleep spindle density during non-rapid eye movement stage 2 (nREM2) sleep, time spent in slow wave sleep (SWS), and slow wave activity (SWA) during SWS have all been associated with the nap-benefit on memory (Kurdziel et al., 2018; Kurdziel et al., 2013; Lokhandwala & Spencer, 2020). Children who benefit more from a mid-day nap also display more sleep spindles, spend a greater portion of time in SWS, and experience more slow wave activity across that nap.

During early childhood sleep architecture also undergoes developmental changes. Specifically, previous work shows that from infancy through early childhood, children spend a smaller portion of time in REM sleep and a larger portion of time nREM sleep (Knoop et al., 2020; Roffwarg et al., 1966). Importantly, afternoon naps during early childhood consist primarily of nREM sleep and contain little to no REM sleep (Jones & Spencer, 2020; Kurdziel et al., 2013; Mantua & Spencer, 2017). Research also demonstrates that there are developmental changes within nREM sleep, including a decrease in time spent in SWS and an increase in time spent in nREM2 sleep and nREM1 sleep (Kurth et al., 2016; Ohayon et al., 2004). In addition to changes in architecture, there are age-related differences in microstructure during early childhood. Between infancy (e.g., 3 months of age) and preschool (e.g., 3 years of age) spindle density declines. After 3 years of age it then begins to increase again through adolescence (i.e., it shows an inverted U-shaped trajectory; Gennaro & Ferrara, 2003; Scholle et al., 2007). Average spindle length also follows a similar inverted U-shaped trajectory, decreasing from infancy to age 3 years and then increasing into adolescence (Scholle et al., 2007). Previous work has also demonstrated that SWA and theta power show age-related decreases, whereas sigma power shows age-related increases from age 2 to 5 years (Kurth et al., 2016).

Taken together, these findings imply that habitual nappers require more regular sleep intervals to consolidate memory, and that developmental changes in sleep architecture and sleep microstructure may partially underscore these differences. However, while there are age-related difference in sleep architecture and sleep microstructure, research has not yet investigated differences in these sleep-

related structures based on nap status. Furthermore, it is also unclear why habitual nappers need to consolidate memories more often than non-habitual nappers.

Brain Maturation and Sleep

Brain maturation is a potential mechanism that may explain why habitual nappers need to offload memories to the cortex more regularly (Lam et al., 2011). Specifically, less mature memory structures may lead to a greater need for consolidation. Furthermore, changes in sleep architecture and microstructure may be related to brain maturation that gives rise to more adult-like consolidation patterns. In other words, it is possible that changes in sleep precede changes in the brain. Specifically, previous work suggests that both REM sleep and nREM sleep play important, but distinct roles in brain maturation (Knoop et al., 2020; Molnár et al., 2019). Namely, REM sleep is believed to provide endogenous activity that leads to maturation of functional networks during infancy. In contrast, SWA during SWS is believed to enhance neural networks later in development via synaptic pruning and down scaling (Knoop et al., 2020; Kurth et al., 2010). Furthermore, sleep spindles during nREM2 sleep have been associated with strength of thalmocortiocal projections (Bonjean et al., 2011). These findings suggest that changes in sleep microstructure and architecture occur before changes in brain maturation that drive the transition to monophasic sleep.

One brain region that is particularly important to memory, and therefore to this investigation, is the hippocampus. Research has demonstrated that episodic memory improvements during early childhood are supported by protracted hippocampal development. Furthermore, some research has demonstrated differences

in hippocampal volume based on parent-reported measures of nap status (Riggins & Spencer, 2020). However, research examining associations between hippocampal maturation, sleep architecture, and sleep microstructure during early childhood is limited. Importantly, the hippocampus is a non-homogeneous structure that can be subdivided into anatomical subregions that include left and right hippocampal head, body, and tail. These subdivisions of the hippocampus show specialization of function (Poppenk & Moscovitch, 2011) and differential relations with memory performance that vary with age (e.g., DeMaster et al., 2013; Riggins et al., 2015; Riggins et al., 2018). Some research suggests that relations between episodic memory in early childhood and hippocampal volumes are quadratic, meaning that for younger children a larger hippocampus is better, while in older children a smaller volume is better (Canada et al., 2019; Riggins et al., 2018). This work demonstrates that bigger is not always better and that during some developmental periods smaller may be superior.

Present Study

In summary, research suggests that sleep supports memory consolidation and that there are marked memory differences between children who nap and those who do not. Furthermore, just as children are transitioning out of their nap, they demonstrate improvements in memory performance that is reliant on the hippocampus (Riggins, 2014). Additionally, during this same developmental period, sleep physiology changes as a function of age (Kurth et al., 2016; Ohayon et al., 2004; Scholle et al., 2007). Furthermore, some work suggests that developmental changes in sleep physiology influence brain maturation (Bonjean et al., 2011; Kurth

et al., 2010a; Molnár et al., 2019). However, relations between the hippocampus, sleep physiology, and memory during early childhood remain unexplored. The purpose of this study is to fill this gap by exploring relations between sleep, memory, and brain development during early childhood to better understand how these variables effect the transition from biphasic to monophasic sleep.

Aim 1 of this investigation was to explore relations between sleep and memory as well as nap status and sleep physiology during early childhood. Findings were hypothesized to replicate the original conclusions from Kurdziel et al. (2013) that mid-day naps would benefit all children but that naps will be more important for habitual nappers' memory than non- habitual nappers' memory. **Hypothesis 1A:** Specifically, it was hypothesized that regardless of nap status, all children would show significantly higher recall scores after the sleep session compared to the wake session. **Hypothesis 1B:** In addition, we hypothesized that memory in habitual nappers would be impaired when made to stay awake. Explicitly, if our predictions were correct, habitual nappers would display significantly lower adjusted wake change scores than non- habitual nappers after a wake session. **Hypothesis 1C:** It was hypothesized that a change in memory score across the nap would be positively associated with sleep spindle density, portion of time spent in nREM2 sleep, and SWS. **Hypothesis 1D:** Additionally, it was predicted that there would be group differences in sleep spindle density, the proportion of time spent in NREM2, and in the proportion of time spent in SWS sleep.

Aim 2 of this investigation was to explore relations between sleep and brain development, specifically hippocampus, and to understand differences in the brain

between habitual nappers and non-habitual nappers. **Hypothesis 2A:** It was hypothesized that there would be relations between hippocampal volumes sleep spindles, the proportion of time spent in NREM2, and the proportion of time spent in SWS sleep, even after controlling for potentially confounding variables like age and sex. **Hypothesis 2B:** Furthermore, it was hypothesized that there would be differences in hippocampal subregion volumes between nappers and non-nappers. However, we did not have specific hypothesis for either of these predictions about which subregions would show group differences or relations due to variability in previous memory/hippocampus relations (e.g., DeMaster et al., 2013; Riggins et al., 2015; Riggins et al., 2018;).

Chapter 2: Methods

Participants

All participants used in this analysis were typically developing children recruited primarily from the Baltimore-Washington area through the Infant and Child Studies Consortium at the University of Maryland (UMD), word of mouth, community advertisements and events. Exclusion criteria for this study include a history of head trauma; any kind of abnormal circadian function; a history of brain abnormality, neurological disorder, psychiatric disorder, developmental delay, or learning disability; a family diagnosis or history of autism spectrum disorder; a history of premature birth (<35 weeks).

Participants for this cross-sectional examination were 51, 3 to 5-year-old children taken from a larger longitudinal study examining the effect of napping on memory and the brain during early childhood. This larger longitudinal study included three time points. The data for the first timepoint was collected when the child was between 3 and 5 years of age. At this initial timepoint the child and was still considered (by parent report) a habitual napper. The data for the second timepoint was collected 6 months later, and data for the third timepoint was collected 6 months after the second timepoint. Importantly, in our initial pre-registration we had planned to take all participants for this cross-sectional examination from the second timepoint of the longitudinal study. The reason we originally selected this timepoint is because we predicted that approximately one third of the participants would still be habitual nappers, one third would be semi-habitual nappers, and one third would no longer be napping. In contrast, we predicted that the first timepoint would consist of mostly

habitual nappers and the third timepoint would consist of mostly non-nappers. These timepoints would not allow us to assess group differences. Unfortunately, due to the COVID-19 pandemic, we were required to stop data collection when only 21 participants had provided usable data at timepoint two. Therefore, we decided to create a cross-sectional dataset from all three timepoints where we only used data for each participant at one of the three timepoints in order to increase the sample size.

Of the 51 children included the data for 27 participants was collected at timepoint one, the data for 22 participants was collected at timepoint two, and the data for 2 participants was collected at timepoint three. The timepoint we selected was based on the timepoint when the child provided usable MRI data. If a participant had MRI data at two timepoints, preference was given to timepoint two to mitigate age differences between nap status groups. If a child had MRI data at timepoint one and timepoint three, but not timepoint two, preference was given to timepoint three to increase the number of non-nappers, a group that was under-represented in our data (since all children were enrolled when they were habitual nappers). When a participant did not provide any usable MRI data, the timepoint was selected based on the availability of PSG data. When a child provided usable PSG data at numerous timepoints, timepoint preferences were the same as described for MRI data.

In this sample, 9.8% of parents identified their child's ethnicity to be Hispanic or Latino regardless of race, 86.3% identified themselves as not Hispanic or Latino regardless of race, and 3.9% did not wish to disclose their child's ethnicity. Furthermore, 60.8% described their race as Caucasian, 15.7% as Black or African American, 3.9% as Asian, and 5.9% as Multiracial. Additionally, 3.9% of parents did

not wish to disclose their child's race. This sample primarily included middle-to high-income households (median = >$155,000, range = < $15,000 - >$195,000) with 3.9% choosing not to disclose their income. Additionally, 80.4% of the sample had at least one parent who achieved a four-year college degree.

Participants were classified into one of three nap status groups based on actigraphy or parent report. Those groups were habitual nappers, semi-habitual nappers, and non-nappers. Consistent with previous work, a habitual napper was a child who napped approximately 5 or more days per week, while a non-napper is a child who napped less than approximately 2 days per week (Desrochers et al., 2016; Kurdziel et al., 2018; Kurdziel et al., 2013). Since our participants were selected from several waves of a longitudinal study, some participants were napping 3-4 days per week. Because we collected data on enough of these children (~33% of the sample), we created a semi-napper group. Specifically, given these criteria, we collected data on 19 habitual nappers, 17 semi-habitual nappers, and 15 non-nappers. However, because not all participants provided usable memory data, PSG data, and brain data (see Table 1), we decided to maximize our sample by utilizing all possible data points, regardless of whether a child had all three types of data (see Table 1).

Table 1

Descriptive Statistics Based on Nap Status Group

Nap Status	**Range$_{age}$**	**N(Females)**	**N$_{PSG}$**	**N$_{memory}$**	**N$_{hippocampus}$**
Nappers	3.4 - 4.96	19 (12)	19	14	10
Semi-Nappers	3.18 - 5.80	17 (11)	14	15	13
Non-Nappers	3.21 - 5.32	15 (5)	12	12	15
Total	3.18 - 5.80	51 (28)	45	41	38

Note. N_{PSG}, N_{memory}, and $N_{hippocampus}$ represent usable data for PSG, the memory task, and hippocampal volume based on nap status inclusive of outliers. Importantly, 1 outlier was removed from total hippocampus, hippocampal head, and hippocampal body analysis (final N=37). Additionally, 2 outliers were removed from hippocampal tail analysis (final N=36). See Table 2 for mean age.

Due to the complex nature of the variables assessed in this study, data was lost for a variety of reasons. Specifically, of the 51 participants included in this analysis, only 45 participants provided usable PSG data, only 41 provided usable memory data, and only 38 provided usable MRI data. Specifically, PSG data was typically lost for one of two reasons; either participant reluctance to fall asleep or a technical error with the PSG recording device. In contrast, almost every child provided memory data. However, children who did not sleep during their nap session were excluded from analysis (N=1). Moreover, MRI data was lost for two reasons; either due to movement in the scanner (N=4) or a refusal to scan (N=5). Importantly, any data loss that does not fit one of the above categories was lost due to the onset of the COVID-19 pandemic (N=4).

Before data collection, all methods were approved by the University of Maryland Institutional Review Board. At the conclusion of each wave, participants received age-appropriate brain prizes (e.g. a brain t-shirt) and parents/guardians received monetary compensation.

Procedure

Each participant partook in two home visits (one home visit was nap session, one was a wake session, counterbalanced for order) and an MRI session. During both the nap and wake home visits, participants first completed an encoding phase of a visual spatial memory task where they were asked to remember the location of images on a grid. Following the encoding phase, children were asked to recall the location of the images in the immediate recall phase. This is where the two visits diverged. During the nap visit, each child was fitted with 14-channel PSG recording montage and encouraged to partake in their typical (or previously typical) nap routine. Conversely, during the wake visit, each child played quietly with non-stimulating toys for the same amount of time that they would typically nap. After the wake period, children who were provided an opportunity to overcome sleep inertia during the nap session were required to sit for ~20 minutes to ensure the wake session was similar to the nap session. Each session concluded with the child completing the delayed recall phase. Approximately 14 days following the first home visit, participants partook in an MRI scan.

Importantly, approximately halfway through the present study, we began to suspect that data from our memory task was not replicating findings from the previous study (Kurdziel et al., 2013). Upon closer examination, we discovered that

the timing of experimental events varied between the two studies. Specifically, children in the original study, performed encoding and immediate recall two hours before their typical nap time. Additionally, children were provided with a full 20 minutes to overcome sleep inertia. In the present study children performed encoding and immediate recall immediately before their typical nap time. They were also, only allotted 5 minutes to overcome sleep inertia. Although we were constrained because we were working in the families' homes, we made changes to ensure the timing was more similar to Kurdziel et al., 2013. Specifically, we increased the amount of time children were provided to overcome inertia by 20 minutes and we increased the time between immediate recall and the experimental condition by 45 minutes. In the present analyses, I created a dichotomous variable called timing to try to account for any difference this manipulation may have caused. However, consideration of the manipulation itself is beyond the scope of my proposed analyses. Future analyses may explore the extent to which this timing difference impacted memory in a more continuous fashion.

Materials

Visual Spatial Memory Task

Stimuli. The memory task used for this study was adjusted from Kurdziel et al. (2013). It consisted of several cartoon images arranged in a grid formation. We attempted to account for celling and floor effects by providing an age appropriate number of stimuli. Children younger than 48 months received a 3x3 grid, children between 48 and 56 months received a 4x3 grid, and children older than 56 months

received a 4x4 grid. The number of stimuli were adjusted during administration as needed (see Allard et al., 2019).

Task. The task included three phases encoding, immediate recall, and delayed recall. During encoding, children identified each image on the grid by name, then the images were hidden, and the child was asked to identify the location of the images. During this phase, children received visual and verbal feedback on their performance. Participants needed to reach a 70% threshold before advancing to the next phase. If a participant cannot meet the 70% threshold within five encoding cycles, they dropped to a smaller grid. During immediate and delayed recall, children were asked to identify the location of the images they saw during encoding, except they did not receive visual or verbal feedback. Consistent with previous work, children were excluded from analysis if they scored 100% at immediate recall to avoid ceiling effects (N=10, including 7 during the sleep session and 3 during the wake session; see final N's in Table 1). Performance was assessed across the nap as adjusted change in nap recall score [(Memory score after the nap – Memory score before the nap)/ Memory score before the nap] and across the wake session as adjusted change in wake recall score [(Memory score after wake – Memory score before wake)/ Memory score before wake]. This measure assesses change in recall and accounts for differences in the number of presented stimuli.

Polysomnography. A 14-electrode montage was used to assess sleep stages and spindles. The montage included two EOG (right and left ocular canthus) leads, two chin EMG leads, and 10 cortical EEG leads. All electrodes were referenced to Cz. Sleep stages were characterized using guidelines from the revised American

Academy of Sleep Medicine manual (AASM, 2007). Sleep spindles will be detected at C3 by a trained researcher using Embla REMLogic software and then verified by a second coder. Spindle density will be examined using Brain Analyzer and an in-house MATLAB code.

MRI Data Acquisition. During one of the home visits, participants were allowed to experience the scanner environment using a fabric tunnel and an audio track. Children were also read a book that explained the purpose of the scanner and the events that would unfold during their scan. At the Maryland Neuroimaging Center, participants then complete training in a mock scanner prior to MR data acquisition. These steps ensured participants were acclimated to the scanner environment and allowed experimenters to provide motion feedback before scanning. Participants were then scanned in a Siemens 3.0-T scanner (MAGNETOM Trio Tim System, Siemens Medical Solutions, Erlangen, Germany) using a 32-channel coil. Structural data was collected using a high-resolution T1 magnetization-prepared rapid gradient-echo (MPRAGE) sequence consisting of 176 contiguous sagittal slices (.9 mm isotripc; 1900 ms TR; 2.32ms TE; 900ms inversion time; 9° flip angle; pixel matrix= 256 x 256). Hippocampal volumes were acquired using Freesurfer v6.0 (surfer.nmr.mgh.harvard.edu; Fischl, 2013) and adjusted using ASAT (nitrc.org/projects/segadapter; Wang et al., 2012). The hippocampus was divided into subregions using standard anatomical landmarks (Riggins et al., 2015).

Actigraphy. At the onset of the study each participant was provided with a pre-programmed actigraphy watch and instructed to wear the watch continuously for

two weeks. Actiwatch data was scored using Philips Respironics following standardized protocols (Acebo et al., 2005). Event markers were used to verify sleep onset and offset. If the participant was missing event markers, or the event markers were not within 20 min of each other, sleep onset and offset was determined manually. Nap status was primarily assessed by dividing the total number of days napped by the number of days the watch was reliably worn, then multiplied by 7. If actiwatch data was unavailable or the watch was worn for less than 3 days, parent report was used.

Questionnaires. Of the 51 participants that took part in this study, 42 provided usable actigraphy data. If actigraphy was unavailable, nap status was assessed using parent report measures. Reports were used in the following hierarchical order; A sleep diary (N=6), an in-house nap-transition questionnaire (e.g. "How many days a week does your child nap?"; N=3), and an over the phone interview (e.g. "Does your child nap?"; N=0). The sleep diary was evaluated first because it provides the most detailed and reliable assessment of each child's nap status. It was administered during the first in-home visit. It required the parent to record all sleep bouts over the two-week period that testing occurred and the average number of napping days was calculated using the same method described for actigraphy. If a child was missing the sleep diary and actigraphy, the nap-transition questionnaire was used. Likewise, if the nap transition questionnaire was missing, the over the phone interview was used. At a

minimum, all caregivers answered this interview question because it was prerequisite to scheduling.

Data Analysis

These hypothesis, methods, and data analytic plan were pre-registered with the Open Science Framework on April 3rd, 2020. The pre-registration has been embargoed until the completion of this thesis, however, it will be available here https://osf.io/ujp2m when lifted. Importantly, there were several minor differences between the present investigation and the published pre-registration. Specifically, for hypothesis 1D, we had originally predicted that non-nappers would spend more time in SWS. Upon, further investigation we changed the direction of this hypothesis to reflect that non-nappers would likly spend less time in SWS (see Kurth et al., 2016). Furthermore, as mentioned previously, due to the impacts of the COVID-19 pandemic the sample used for this investigation is smaller than anticipated and uses data from all three waves of the longitudinal study. Additionally, the original pre-registration did not anticipate differences in conditioning timing that was used as a dichotomous covariate in some analysis. Finally, the following method for identifying outliers and the method for identifying multicollinear variables was also not included in the original pre-registration.

Previous work has indicated that ANOVA's are sensitive to extreme outliers (Osborne & Overbay, 2004). Specifically, research indicates that outliers near or above 3 standard deviations away from the grand mean may significantly influence results. Furthermore, these outliers may mask true effects, even when they are naturally occurring in the population (Barnett & Lewis, 1994). Therefore, before

analysis, outliers greater than 2.5 standard deviations away from the grand mean were removed (see Table 1).

Before addressing the main hypothesis, the effects of counterbalancing (i.e., nap or wake session fist) on memory performance were assessed using a one-way ANOVA. Specifically, differences in adjusted memory change scores between the home sessions were examined. Moreover, we examined group differences in age, sex, timing, and ICV based on nap status using a one-way ANOVA for age and ICV, and a chi-square test for timing and sex. If significant differences were found for condition age, sex, timing, or ICV, they were controlled for in the appropriate analysis (e.g., ICV in brain analysis and timing in memory analysis). Given that these guidelines could lead to an extensive list of covariates, VIF analysis was used to assess multicollinearity of variables. If a variable had a VIF > 2, it was removed from the analysis.

To address the first aim regarding differences in memory performance based on condition (e.g., nap session vs. sleep session) and nap status (e.g., habitual napper, semi-habitual napper, and non-napper), a two-way 2x3 Mixed ANOVA controlling for age, sex, and timing was conducted **(hypothesis 1A and hypothesis 1B)**. To examine post-hoc comparisons a series of pairwise t-test were conducted. Additionally, we examined relations between sleep physiology and memory using a regression model controlling for age, sex, and timing (**hypothesis 1C**). In the first analysis we assessed relations between adjusted change in nap score and sleep spindles. In a second analysis, we assessed relations between adjusted change in nap score and proportion of time spent in nREM2 sleep. Finally, in a third analysis, we

assessed relations between adjusted change in nap score and proportion of time spent in SWS sleep. Finally, to address group-based variation in sleep, we examined differences in sleep spindle density, the portion of time spent in nREM2 sleep, and the percentage of time spent in SWS using sperate one-way ANCOVA's controlling for age and sex (**hypothesis 1D**). For all ANCOVA's, Scheffe test were conducted to assess post-hoc comparisons. The Scheffe test was chosen because it is the most conservative option for post-hoc testing with ANCOVA's.

The second aim of this study was to understand relations between the hippocampus and sleep. To assess relations between sleep and the hippocampus, we conducted a regression model comparing total hippocampal volume, hippocampal subregion volumes, and sleep spindle density across the afternoon nap (**hypothesis 2A**). We also conducted these same models using portion of time spent in nREM2 sleep and SWS. Furthermore, we examined differences in hippocampal volume, both subregion and the total structure, based on nap status (**hypothesis 2B**). Specifically, we used separate one-way ANCOVA's for each volumetric variable, starting with the total hippocampus, then bilateral hippocampal head, body, and tail independently. If differences were significant in a bilateral subregion, lateralized differences in that subregion were examined. When significant differences were identified post-hoc Scheffe tests were conducted.

Chapter 3: Results

Outlier Identification

Using the previously described criteria for identifying outliers, two data points were removed from PSG analyses, one data point was removed from total hippocampal volume and hippocampal body volume, and two data points for hippocampal tail volume. (Note: one participant contributed to the outliers in total, body, and tail, this participant did have notable motion artifacts (e.g., banding) in their structural scan that made manual edits necessary and difficult).

Preliminary Analysis

Due to unanticipated data loss due to the COVID-19 pandemic this investigation may be underpowered. Therefore, we will address both marginal ($p < .10$) and significant ($p < .05$) findings. Preliminary analyses examined differences based on timing and order. Preliminary results revealed that there were no order effects on memory. Specifically, the order of sessions was evenly distributed based on nap status, χ^2 (2, N = 49) = 0.015, $p = .99$, and memory change scores did not differ based order of session, $F(1, 89) = 0.044$, $p = .88$. Importantly, while there were no significant differences in memory score based on timing, $F(1, 89) = 0.824$, $p = .37$, there was a marginal difference in timing based on nap status χ^2 (1, N = 49) = 5.19, $p = .07$. Specifically, non-nappers were more likely to experience timing that did not allow for decay after encoding and did not adjust for sleep inertia. Therefore, timing will be controlled for in all memory analysis.

Differences between nap-status groups in age, sex, and ICV were also assessed (see Table 2). These findings revealed no significant differences in sex, χ^2 (1, N = 49) = 4, p = .14, or ICV, $F(2, 35) = 1.55$, p = .23. However, there were differences between the groups in age, $F(2, 48) = 4.57$, p = .02. Post-hoc tests demonstrated that non-nappers were older than habitual nappers but neither differed from semi-habitual nappers. Therefore, all analyses that assess group differences will include age as a covariate. Moreover, although sex and ICV were not significantly different across nap groups, bivariate correlations (Table 3) showed that there were moderate associations with memory, sleep spindles, and hippocampal volumes. Importantly, the field also suggests that these are potentially confounding variables, therefore, they will be included as covariates in appropriate analysis regardless.

Table 2.

Means, Standard Deviations, and Group Differences Based on Nap Status

	Napper		**Semi-Napper**		**Non-Napper**		**F value**
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	
Covariates							
Age (years)	3.95	0.42	4.10	0.57	4.55	0.77	**4.568***
ICV (mm^3)	1237189	99813	1250265	102000	1306026	99150	1.553
Sleep							
Spindle Density	0.46	0.43	0.56	0.44	0.46	0.24	0.004
nREM2%	27.89	13.17	38.42	13.93	34.73	6.96	**2.754†**
SWS%	59.12	19.16	44.97	12.99	52.83	12.04	**2.971†**
Spindle Counts	9.89	5.91	18.7	17.8	12.9	8.37	0.260
nREM (mins)	29.3	16.5	34.7	14.7	28.9	9.68	0.680
Memory							
Memory Change Score	-0.005	0.258	-0.111	0.334	-0.145	0.341	**.225 †**
Sleep Change Score	0.034	0.264	-0.069	0.361	-0.156	0.373	1.171
Wake Change Score	-0.040	0.256	-0.150	0.320	-0.136	0.322	2.072
Hippocampus							
Total (mm^3)	5752.56	502.92	5689.00	370.61	6177.42	471.08	**3.788***
Head	3042.50	365.01	2850.88	368.68	3301.00	413.67	**3.481***
Body	1888.00	248.42	2028.38	284.55	1986.50	195.64	0.475
Tail	884.38	167.06	809.75	206.52	889.92	209.19	0.678

$^{\dagger}p<.10$. $^{*}p < .05$. $^{**}p < .01$. $^{***}p < .001$.

Note. Spindle counts and nREM (mins) are used to calculate spindle density (e.g., Spindle Counts/total time spent nREM2).

Table 3

Correlation Matrix: Assessment of Potential Confounding Variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Demographics															
1. Sex															
2. Age	0.34														
3. Nap Status	0.29	0.28													
4. Timing	-0.21	-0.30	-0.32												
Memory															
5. Memory Change Score [a]	-0.24	-0.19	-0.25	0.04											
6. Sleep Change Score	-0.31	-0.08	-0.12	-0.03	0.61	1.00									
7. Wake Change Score	-0.17	-0.30	-0.40	0.10	0.59	0.21									
PSG															
8. Spindle Density	-0.10	-0.07	-0.31	0.07	0.05	0.05	0.05								
9. nREM2% [b]	0.10	0.25	0.22	-0.20	-0.26	-0.29	-0.23	-0.59							
10. SWS% [c]	0.19	0.08	-0.11	-0.14	-0.11	-0.19	-0.02	0.31	-0.52						
Brain															
11. Total Hippocampus	0.51	0.38	0.45	0.06	-0.18	-0.16	-0.20	-0.37	0.09	0.18					
12. Head	0.53	0.25	0.33	-0.19	-0.11	-0.18	-0.04	-0.32	-0.01	0.37	0.81				
13. Body	-0.04	0.36	0.13	0.36	-0.09	0.05	-0.25	0.00	0.11	-0.32	0.11	-0.42			
14. Tail	0.14	-0.14	0.21	0.08	-0.07	-0.06	-0.09	-0.21	0.11	0.04	0.57	0.34	-0.16		
15. ICV	0.67	0.39	0.28	-0.3	-0.19	-0.08	-0.3	-0.07	0.25	0.08	0.38	0.25	0.36	-0.14	

Note. a = memory scores collapsed across both the nap session and the wake session.

b = portion of time spent in nREM2 sleep. c = portion of time spent in SWS.

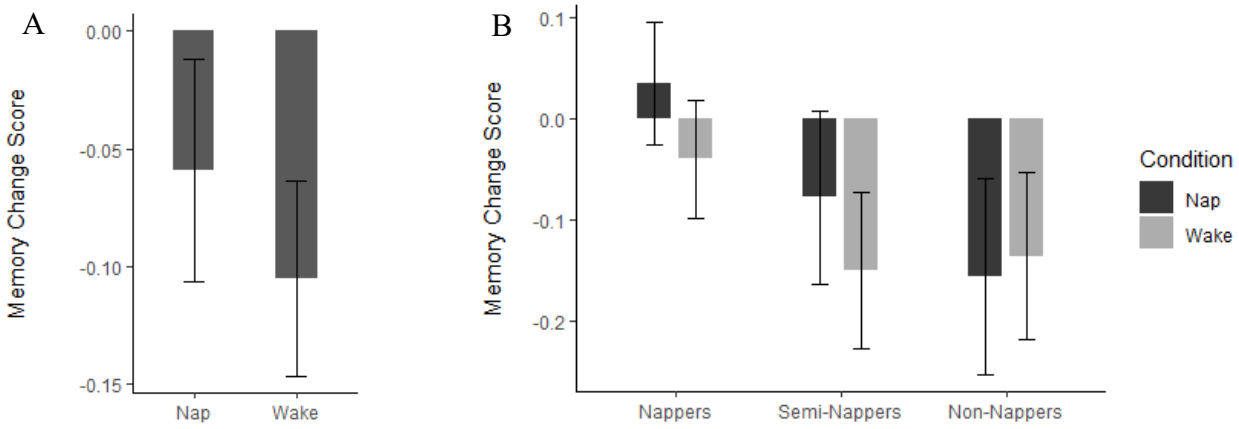
Memory

Results from the 2x3 Mixed ANCOVA, examining memory differences based on nap status and condition, controlling for age, sex, and timing, (**hypothesis 1A** and **hypothesis 1B**) indicated a significant main effect for nap status, $F(2, 34) = 3.71$, $p = .035$, and a marginal main effect for condition, $F(1, 34) = 4.009$, $p = .053$ (Figure 1A). Specifically, as illustrated in Figure 1A, the nap condition showed less decay than the wake condition. To explore the main effect of nap status group, a series of post-hoc pairwise t-tests was conducted to examine between group differences. Results suggested that there was a marginal difference between nappers and non-nappers on the memory change score, such that non-nappers experienced more memory decay across the testing condition than did habitual nappers (Figure 1B). Results suggested that there was a marginal difference between nappers and non-nappers on the memory change score, such that non-nappers experience more memory decay across the testing condition than did habitual nappers (Figure 1B). There was no interaction between nap status and condition (see Figure 1B).

To examine associations between sleep measures and nap change score (**hypothesis 1C**) three general linear regression models were conducted controlling for age, sex, and timing. The models examined associations between sleep change score, spindle density, $F(4, 34) = .318$, $p = .86$, time spent in nREM2 sleep, $F(4, 35) = .531$, $p = .714$, and time spent in SWS, $F(4, 36) = 1.41$, $p = .353$. Results demonstrated that all three models were not significant.

Figure 1

Differences in Memory Change Score Based on Nap Status and Condition



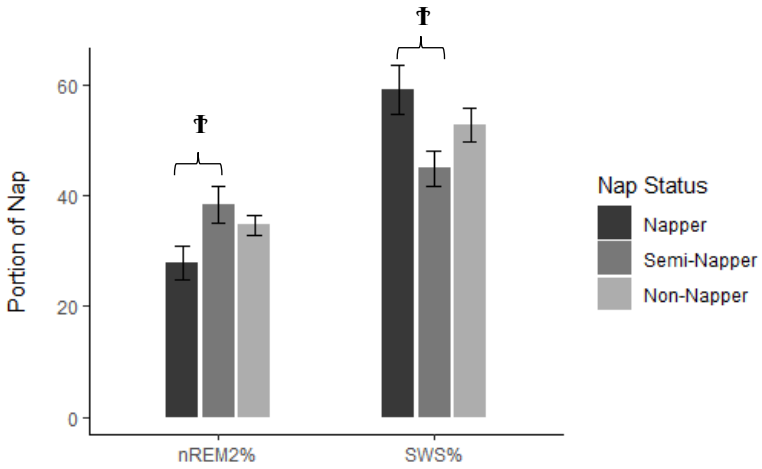
Note: A) Represents the main effect for condition on memory change score B) Represents memory change score based on nap status and condition.

Sleep Physiology

To address differences in spindle density, portion of time spent in nREM2 sleep, and portion of time spent in SWS based on nap status (**hypothesis 1D)**, three separate one-way ANCOVA's were conducted controlling for age and sex. Results demonstrated a marginal difference in portion of time spent in nREM2, $F(2, 40) = 2.75$, $p = .07$, and in portion of time spent in SWS , $F(2, 40) = 2.97$, $p = .06$ sleep, but not spindle density. Specifically, findings suggest that semi-habitual nappers spend a greater portion of time in nREM2 and in SWS across the nap compared to habitual nappers (Table 2; Figure 2).

Figure 2

Differences in Sleep Architecture Based on Nap Status and Condition



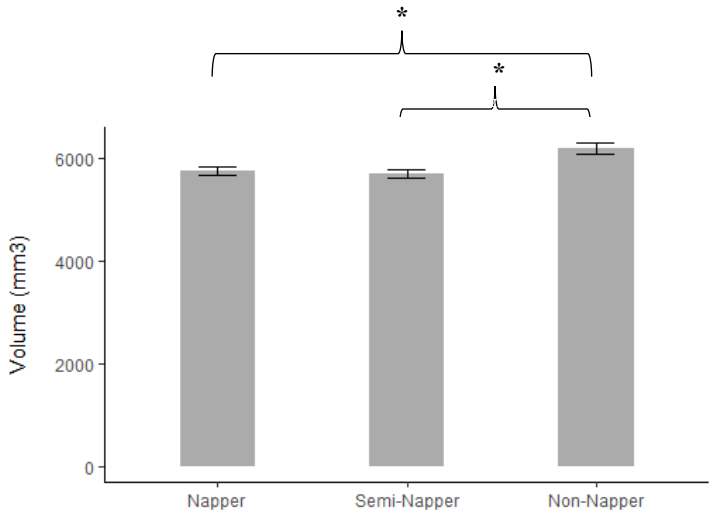
†$p<.10$.

<u>*Hippocampus*</u>

A one-way ANCOVA was conducted to assess difference in total hippocampal volume based on nap status controlling for age, sex and ICV (**hypothesis 2A**). Findings demonstrate that there were significant differences in hippocampal volume based on nap status, $F(2, 31) = 3.79$, $p = .03$. Specifically, non-nappers had a larger total hippocampus than both nappers and semi-nappers (Figure 3).

Figure 3

Differences in Total Raw Hippocampal Volume Based on Nap Status

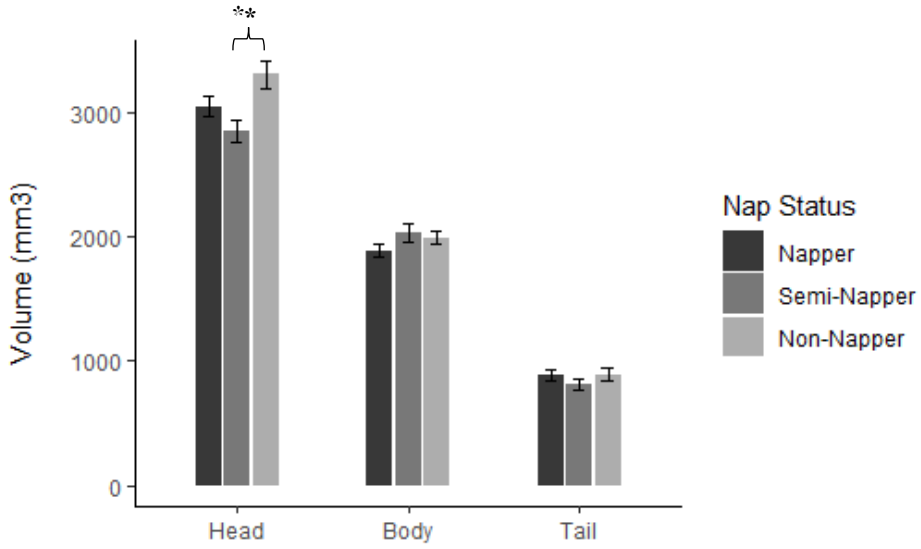


*$p < .05$.

Three additional one-way ANCOVA's, were conducted to assess whether there were group differences in hippocampal subregions controlling for age, sex, and ICV (**hypothesis 2A**). The analysis revealed that there were significant differences in hippocampal head, $F(2, 32) = 3.48$, $p = .04$, but not body or tail, ps $> .10$. Specifically, non-nappers had a larger hippocampal head on average than semi-habitual nappers. Additionally, two follow up one-way ANCOVA's demonstrated that group differences were marginal in right hippocampal head, $F(2, 32) = 3.08$, $p = .06$, and significant in left hippocampal head $F(2, 32) = 3.39$, $p = .046$. Post-hoc testing revealed that in right hippocampal head, semi-habitual nappers were larger than habitual nappers and in left hippocampal head, non-nappers were bigger than both habitual nappers and semi-habitual nappers (Figure 4).

Figure 4

Differences in Hippocampal Subregions Volumes Based on Nap Status



*$p < .05$.

Finally, a linear regression was used to examine the association between sleep physiology (e.g., spindle density, nREM2%, and SWS%) and hippocampal volume (**hypothesis 2B)**. Findings show that there was no significant association with spindle density (see Table 4; **β**=-3.3e+02, p=0.17). Moreover, the association between sleep spindle density and hippocampal subregion volumes, along with all other associations between the hippocampus, portion of time spent in nREM2 sleep, and portion of time in SWS were non-significant when controlling for age, sex, and ICV.

Table 4

Regression Table: Relations between Sleep Physiology and Total Hippocampal Volume

Predictor Variable	Spindle Density	nREM2%	SWS%
	β	*β*	*β*
Intercept	**5170.00*****	**5375.00*****	**4847.00*****
Total Hippocampus	-326.30	-2.85	4.61
Age	-138.40	-192.10	-139.90
Sex	**390.10**†	**423.70** †	352.60
ICV	1.06E-03	1.01E-03	1.01E-03
Adj. R^2	0.27	0.21	0.22
F value	**3.91***	**3.01***	**3.13***

†$p<.10$, *$p < .05$, **$p < .01$, ***$p < .001$.

Chapter 4: Discussion

The purpose of this study was to explore relations between sleep, memory, and brain development during early childhood to better understand how these variables are connected to the transition from biphasic to monophasic sleep. Furthermore, this study aimed to build on previous findings from Kurdiziel et al., 2013, that demonstrated differences in memory across an afternoon nap based on nap status that were related to spindle density. Specifically, the present examination was designed to replicate and extend these findings using a similar design but also examining differences in hippocampal volume and sleep physiology based on nap status. Results from the present investigation were similar to the previous report because findings replicated differences in memory performance between nap and wake conditions. Interestingly, although we did not replicate previous memory differences between habitual nappers and non-nappers, we did identify marginal differences between these groups in both sleep architecture and hippocampal volume. The goal of this section is to discuss these findings, consider limitations, and explore important points for future research.

Memory

Consistent with **hypothesis 1A** that all children would show significantly higher recall scores after the sleep session compared to the wake session, participants in the nap condition performed significantly better on the visual-spatial memory task than participants in the wake condition, regardless of nap status (Figure 1A). These findings suggest that an afternoon nap is beneficial for everyone, even those who are

not habitual nappers. These findings are consistent with previous work examining differences in memory performance between a nap session and wake session during early childhood (Kurdziel et al., 2018; Kurdziel et al., 2013; Lokhandwala & Spencer, 2020). Specifically, past work has shown that both habitual nappers and non-nappers benefit from an afternoon nap, but habitual nappers' memory is significantly more impaired when they are kept awake.

Contrary to expectations, non-nappers demonstrated lower memory change scores than habitual nappers, regardless of condition. In other words, it appears that non-nappers experienced more memory decay than habitual nappers. This unexpected finding could have been caused by group differences in timing (i.e., the amount of time allotted to overcome inertia and the delay between recall sessions) and increased memory change score variability. Specifically, non-nappers were less likely to have an opportunity to overcome sleep inertia and experienced less time between immediate recall and the condition. For non-nappers, this could suggest that lower delay recall scores after the nap session are a marker of drowsiness and not memory. Additionally, the lack of delay between encoding and the condition created less of an opportunity for there to be nap-benefit on memory due to lack of memory decay prior to the nap. Although, we did control for timing, the dichotomous variable used may not represent the total variance created by these timing differences.

Moreover, contrary to **hypothesis 1B** that predicted habitual nappers would display significantly lower wake change scores than non-habitual nappers after a wake session, we found that there was no interaction between nap status and condition (nap vs. wake). These findings are not consistent with past research that

demonstrated habitual nappers' memory is significantly more impaired when they are kept awake (Kurdziel et al., 2013). Therefore, this finding could have been caused by lack of power due to data loss. However, it is important to note that these findings are consistent with Kurdziel et al., (2018) that found no interaction between condition and nap status on memory change score across an afternoon nap or wake session. Importantly, this previous investigation also demonstrated that an interaction between condition and nap status on memory change score emerged 24 hours after the initial encoding session (Kurdziel et al., 2018). Specifically, memory recall was significantly better for habitual nappers after overnight sleep following a nap session, while memory recall demonstrated significant forgetting following a wake session. There was no effect of the nap or wake session on memory following overnight sleep for non-habitual nappers.

Future studies that examine difference in memory consolidation based on nap status should consider the impact of sleep inertia on memory change across a nap. Studies should also aim to keep the time between immediate recall and delayed recall consistent across all participants, while ensuring there is sufficient time for memory decay between immediate encoding and the nap condition. Additionally, future studies may want to consider using a different memory task. Specifically, this memory task may have poor psychometric properties that do not allow it to capture fine-grained differences between individuals. For example, if a participant received the smallest memory grid, they were only able to accomplish 9 possible scores during immediate and delayed recall. This restricts the outcome range, especially in younger children. Future memory tasks should aim to study other hippocampal dependent

memory abilities like pattern separation and autobiographical memory, preferably with a more precise outcome variable. Finally, future studies should consider including a 24-hour delayed recall phase to assess the effects of an afternoon nap on memory performance during a complete circadian cycle.

Sleep

Contrary to **hypothesis 1C** that predicted nap change scores would be positively associated with spindle density, portion of time spent in nREM2 sleep, and portion of time spent in SWS, there was no association between memory performance and sleep physiology. These findings are not consistent with past literature suggesting that both sleep spindle density and time spent in SWS are associated with nap change score (Kurdziel et al., 2013; Lokhandwala & Spencer, 2020). Importantly, these results could have driven by limitations with the memory task discussed above (e.g., differences in timing and increased delay between recall session for some children). Furthermore, another explanation for these surprising findings is that previous studies that demonstrated associations between sleep spindle density and nap change score reported higher average spindle densities (e.g., M = .96 vs. M = .46; Kurdziel et al., 2013). Moreover, previous work that did not find association between spindle density and nap change score demonstrated an average spindle density that was more similar to the present study (e.g., M = .72; Lokhandwala & Spencer, 2020). Therefore, these differences may have arisen from differences in spindle density estimation. One final explanation, is that nap architecture and microstructure effect memory on a 24-hour cycle. Specifically, Kurdziel et al., (2018) found that relations between memory change score following a nap and memory change score following overnight sleep

were fully mediated by nap microstructure (e.g. Slow Wave Activity). This could suggest that differences in memory performance across an afternoon sleep are supported by sleep microstructure over during both the nap session and during overnight sleep.

Partially consistent with **hypothesis 1D** that predicted there would be group differences in sleep physiology (e.g., spindle density, nREM2%, and SWS%) based on nap status, results indicated that semi-habitual nappers spent a greater proportion of time in nREM2 sleep and less time in SWS compared to habitual nappers when controlling for age and sex. However, there were no differences between non-nappers and habitual nappers, although, results demonstrate that the non-significant differences between these groups are in the same direction (e.g., non-nappers appear spend less time in SWS and more time in nREM2 sleep compared to habitual nappers; Figure 2). This non-significant finding could be a side effect of our small sample size. Therefore, future work should aim replicate these findings using a larger sample. Moreover, future work could benefit from investigating these associations using a continuous variable, like naps/week, that may be better equipped to assess the association between sleep architecture and nap status. Importantly, there were also no differences in sleep spindle density based on nap statues. While previous studies have shown that both SWS and nREM2 sleep undergo a gradual developmental shift during early childhood, to our knowledge this is the first study to demonstrate differences in sleep architecture based on nap status when accounting for age (Gennaro & Ferrara, 2003; Kurth et al., 2016). These findings could suggest that shifts in sleep architecture precede the nap transition and may explain previously

reported differences in memory performance based on nap status (Kurdziel et al., 2018; Kurdziel et al., 2013; Lokhandwala & Spencer, 2020).

Hippocampus

The most novel aspect of this investigation was our ability to investigate hippocampal volumes during early childhood. Consistent with **hypothesis 2B** that predicted there would be differences in hippocampal volume based on nap status, results indicate that non-nappers have a larger total hippocampus compared to both semi-habitual nappers and habitual nappers. Furthermore, these findings appear to be driven by bilateral hippocampal head, and not body or tail. These findings are consistent with a predication by Lam et al., suggesting that cognitive difference based on nap status are driven by differences in brain structure (Lam et al., 2011). Furthermore, these findings are partially consistent with a recent publication that examined hippocampal subfields instead of hippocampal subregions (Riggins & Spencer, 2020). This study revealed that in children aged 4 to 6 years, the CA1 subfield in the hippocampal body was significantly smaller in non-nappers compared to habitual nappers. While these findings are in the opposite direction of the present study, they could be complimentary. Specifically, subfields are embedded within subregions (Insausti and Amaral, 2012; Poppenk et al., 2013). This allows subfields to be differentially distributed based on subregion. Therefore, both a smaller hippocampal CA1 subfield and a larger hippocampal head could be markers of hippocampal maturity. The key takeaway is that both studies show marked differences in hippocampal volumes based on nap status.

Contrary to **hypothesis 2A** that predicted there would be relations between hippocampal volumes and sleep physiology, results demonstrated that hippocampal volumes were not associated with spindle density, proportion of time spent in nREM2 sleep, nor the proportion of time spent in SWS. This is not consistent with previous empirical work suggesting that changes in sleep architecture and microstructure are related to brain maturation (Bonjean et al., 2011; Kurth et al., 2010a; Molnár et al., 2019). This could be due to lack power in our sample. Specifically, our sample size was reduced due to the COVID-19 pandemic and difficulties scanning 3 to 5-year-old children. These findings could also suggest that there is no association between hippocampal volume and sleep physiology.

Broader Impacts

This work has implications for several applied areas including parenting practices, physician-based sleep recommendations, and preschool nap policies. Specifically, this work supports previous research suggesting that taking a nap is beneficial for memory in preschool aged children, regardless of nap status. Furthermore, this study demonstrates that nap status is underscored by differences in brain structure and function. For this reason, day cares, preschools, and other individuals who care for preschool aged children should consider how a nap opportunity may interact with brain development.

Conclusions

In summary, we replicated findings that suggest memory performance across a nap is superior to memory performance across a wake session lasting the same

amount of time (Figure 1A). However, we failed to show an interaction between condition and nap status that has been demonstrated by previous studies (Kurdziel et al., 2013; Kurdziel et al., 2018). These unexpected results may have been caused by differences in the timing for the memory task. Moreover, we did demonstrate that children who are transitioning out of their afternoon nap spend a greater portion of time in nREM2 sleep and a smaller portion of time in SWS when accounting for potentially confounding variables (age, sex, and timing). Furthermore, non-nappers have a larger hippocampal volume than both semi-nappers and habitual nappers. Together, these findings may suggest that differences in memory performance based on nap status reported by previous studies (e.g., Kurdziel et al. 2013) may be related to differences in sleep architecture and hippocampal structure. Given previous research (e.g., Bonjean et al., 2011; Kurdziel et al., 2013 Kurth et al., 2010a; Molnár et al., 2019), this could imply that changes in nap habits are preceded by the development of sleep architecture and hippocampal structure. In essence, as sleep architecture matures, this leads to changes in the brain, allowing for increased memory consolidation (Bonjean et al., 2011; Kurth et al., 2010a; Molnár et al., 2019). Importantly, this study only partially supports this claim. Future work should replicate and expand on these findings.

Given these findings, future research should assess the effects of nap status on memory performance across a 24-hour period. Moreover, studies could benefit from examining the effects of brain development on sleep architecture and microstructure across a 24 hour period during the nap transition. Additionally, future studies should consider differences in network-based markers of neurobiological maturity (e.g.,

hippocampal connectivity) based on nap status. Specifically, previous work has demonstrated that markers of functional correlations between the hippocampus and other memory related regions is associated with longitudinal development of episodic memory during early childhood. Therefore, this work could further illuminate the mechanism that drives children to transition out of their afternoon nap (Geng et al., 2018). Furthermore, it would provide additional insight into how sleep impacts memory and the brain during early childhood.

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