Infant Behavior and Development 65 (2021) 101647

Contents lists available at ScienceDirect

Infant Behavior and Development

journal homepage: www.elsevier.com/locate/inbede

The memory benefits of two naps per day during infancy: A pilot investigation

Gina M. Mason [a,b], Laura B.F. Kurdziel [c], Rebecca M.C. Spencer [a,b,d,*]

[a] Department of Psychological & Brain Sciences, United States
[b] Neuroscience & Behavior Program, University of Massachusetts, Amherst, United States
[c] Department of Psychology, Merrimack College, United States
[d] Institute for Applied Life Sciences, University of Massachusetts, Amherst, United States

ARTICLE INFO

Keywords:
Naps
infancy
declarative memory
slow-wave activity
polysomnography

ABSTRACT

In infancy, sleep occurs in multiple nap and overnight bouts that change developmentally in quantity and distribution. Though studies suggest that infant memory benefits from a single nap, no work has assessed the relative benefits of different naps (morning vs. afternoon), nor how multiple naps support memory across the day. We investigated the memory benefit of a morning nap, relative to morning wake, and the effect of these intervals on afternoon nap function in 9-month-olds (n = 15). Infants participated in two within-subjects conditions (separated by 1-2 weeks). In the Nap-Nap condition, infants took their morning and afternoon naps; in the Wake-Nap condition, infants were kept awake during morning naptime, but napped unrestricted in the afternoon. Before each nap/wake interval, infants completed an imitation memory task, with memory assessed again shortly after the nap/wake interval. In the Nap-Nap condition, infants showed memory retention across morning and afternoon naps. In contrast, infants tended to forget items learned across morning wake in the Wake-Nap condition. Moreover, morning wake was associated with a significant decline in post-nap retention of items learned in the afternoon. Furthermore, relations between nap slow-wave activity (SWA) and memory varied across naps, with SWA either not predicting (morning naps) or positively predicting (afternoon naps) memory change in the Nap-Nap condition, but negatively predicting afternoon memory change in the Wake-Nap condition. We conclude that two naps per day (rather than one) aids memory at 9 months, and that skipping the morning nap may moderate relations between afternoon nap physiology and memory.

1. Introduction

Across early development, infants' sleep patterns change significantly. While infants' 24 -h sleep duration ranges from 12-20 hours, the distribution of sleep throughout the day shifts from a polyphasic pattern at birth (i.e., multiple short naps across the day and night) to a biphasic pattern of one daytime nap and one overnight sleep bout by approximately 15-18 months (Galland, Taylor, Elder, & Herbison, 2012; Weissbluth, 1995). Around 6-12 months, infant sleep typically stabilizes into an intermediary pattern of triphasic sleep, composed of a morning nap, afternoon nap, and overnight sleep (Weissbluth, 1995). However, the possible functional

* Corresponding author at: University of Massachusetts, Institute for Applied Life Sciences, 240 Thatcher Way, S315, Amherst, MA, 01003, United States.
E-mail address: rspencer@umass.edu (R.M.C. Spencer).

https://doi.org/10.1016/j.infbeh.2021.101647
Received 10 June 2021; Received in revised form 1 September 2021; Accepted 7 September 2021
Available online 13 September 2021

significance of the triphasic sleep pattern in early development is unknown.

During their waking hours, infants are immersed in an environment full of new objects, people, and experiences that they must extract information from and learn to respond to appropriately. In response to these demands, infants exhibit remarkable capacities for associative/social learning and memory from an early age (Goldstein, Schwade, & Bornstein, 2009; Hayne, Boniface, & Barr, 2000). While the ability to extract regularities from repeated experiences has been shown in infants soon after birth (Bulf, Johnson, & Valenza, 2011; but see also Slone & Johnson, 2015), infants' ability to remember specific objects, events or experiences based on only a few exposures appears to emerge more incrementally, with some reports suggesting this capacity to be explicitly observable by approximately 6 months (Barr, Dowden, & Hayne, 1996; Bauer, Wenner, Dropik, & Wewerka, 2000; Hayne et al., 2000; Richmond & Nelson, 2007). This ability to remember specific details with limited experience is considered by some to be a hallmark of more advanced forms of declarative memory, involving explicit long-term memory for facts and events (Gómez & Edgin, 2016; Richmond & Nelson, 2007; Squire & Zola-Morgan, 1991; Squire, 2004).

Given the co-occurring changes in sleep patterns and declarative memory development during infancy, recent work has begun to examine whether infant sleep, particularly daytime napping, may aid declarative memory. In one study (Seehagen, Konrad, Herbert, Schneider, & Heller, 2015), researchers probed the benefits of a single nap (whichever nap caregivers preferred) on 6- and 12-month--old infants' declarative memory using a deferred imitation paradigm. In this task, experimenters modeled salient target actions with specific novel objects, and presented the same objects to infants after a delay to observe whether the actions were reproduced. Four hours after initial learning, infants reproduced more target actions than expected by chance if they had napped in between learning and test. In contrast, infants kept awake during the delay did not reproduce more actions than chance. Other declarative memory paradigms (e.g., word-object learning, visual recognition) have also shown to benefit from a nap in infants (for reviews, see Mason, Lokhandwala, Riggins, & Spencer, 2021; Seehagen, Zmyj, & Herbert, 2019), indicating that napping may play a significant functional role in supporting declarative memory in various contexts throughout infancy.

Regarding the specific mechanisms by which naps might support declarative memory, work in adults suggests that certain sleep stages and neural characteristics of sleep may actively assist the relative strengthening and transfer of memories into long-term stores. In particular, the 0.5-4 Hz frequency cortical slow oscillations characteristic of slow-wave sleep (SWS, also known as NREM stage 3) are thought to aid memories via two pathways: 1) a process of global synaptic downscaling, whereby synaptic connections increased during learning are maintained while synaptic "noise" is lost (Tononi & Cirelli, 2006), and 2) a process of active systems consolidation, in which slow oscillations synchronize with sleep spindles (short bursts of 10-16 Hz frequency EEG activity) to promote memory reactivation and information transfer from the hippocampus, a memory-related brain region, to longer-term cortical stores (Diekelmann & Born, 2010). Sleep spindles are thought to be coupled to sharp-wave ripples arising from the hippocampus, and may indicate moments in which reactivation and information exchange is occurring between hippocampus and the neocortex (Born & Wilhelm, 2012; Diekelmann & Born, 2010).

Among infants and children, studies measuring the neural features of naps in relation to declarative memory have provided some support for a role of SWS and spindles. For example, one study in 6.5-month-olds found that nap-dependent retention of previously learned words from an artificial grammar paradigm was related to frontal and central slow-wave activity (SWA), a measure of slow-wave strength (Simon et al., 2017). Additionally, among toddler-aged children (14-17 months), nap-dependent memory for specific object-word pairs was significantly related to sleep spindle activity in frontal areas (Friedrich, Mölle, Friederici, & Born, 2020), whereas for younger and older infants (6-8 months and 14-16 months), memory for more generalized semantic content was related to time in non-REM stage 2 (N2) sleep (in younger infants) as well as centroparietal spindle activity and density across non-REM sleep (Friedrich, Wilhelm, Mölle, Born, & Friederici, 2017; Friedrich, Mölle, Friederici, & Born, 2019). Studies in early childhood (3-5 years) have similarly found relations between nap-dependent visuospatial declarative memory and sleep spindle density (Kurdziel, Duclos, & Spencer, 2013), and between episodic declarative memory and SWS in the nap (Lokhandwala & Spencer, 2020). Although it is not entirely clear why different aspects of N2, SWS and sleep spindles are related to declarative memory tasks, these studies together indicate an active role of sleep, specifically non-REM features, in memory strengthening in infancy.

Despite the recent studies examining how naps and their neural features broadly support memory in infancy, no studies have critically assessed the relative benefits of different nap intervals (morning and afternoon) on infant memory. Previously, studies of young infants either examined infants' first nap bout of the day (e.g., Simon et al., 2017) or the most reliable nap bout for scheduling according to the caregiver (e.g., Friedrich et al., 2017; Seehagen et al., 2015). While such studies have provided crucial insights into the benefits of naps for infants' memory, not systematically exploring the structure of both naps during the triphasic period leaves open the question of whether one nap might be more beneficial for learning than the other, perhaps due to differences in sleep stage architecture or neural microstructure. For example, in adults, some studies have suggested that morning naps contain more rapid-eye-movement (REM) sleep and less SWS than afternoon naps (Groeger, Lo, Burns, & Dijk, 2011; Karacan, Rosenbloom, Londono, Williams, & Salis, 1975). Additionally, one study in young children (2, 3 and 5 years) indicated differences in nap features such as SWS and SWA for naps taken in the morning compared to the evening (Kurth, Lassonde et al., 2016). However, no work has yet examined the sleep physiology differences of multiple naps taken on the same day, particularly during infancy (when multiple nap bouts per day are common). Understanding the structure and memory benefits of different infant nap intervals may thus inform interventions to optimize the benefits of each nap, and may also help to explain previously disparate findings regarding the mechanisms by which child naps are thought to aid memory (see Mason et al., 2021 for review).

Additionally, exploring more broadly whether two naps per day are more beneficial for declarative memory during the triphasic age (Galland et al., 2012; Weissbluth, 1995), as opposed to one unrestricted nap only, may provide insights into the functional importance of this sleep pattern for early cognitive development. Theoretically, if memory networks including the hippocampus have age-dependent computational or storage limitations (Gómez & Edgin, 2016), it may be that more frequent nap bouts are necessary for

information transfer from these immature hippocampal networks to broader cortical areas. On the other hand, multiple nap bouts may simply be an artifact of infants' developing circadian systems or another unobserved process, and may not serve a functional purpose for memory consolidation as compared to one nap alone. Thus, evaluating the effects of one nap as opposed to two for memory will help to elucidate the importance of nap frequency and facilitate evidence-based recommendations regarding optimal nap routines for infants within this age range (Siren-Tiusanen & Robinson, 2001).

In the present study, we aimed to clarify how morning and afternoon naps may differ physiologically and how they work together to support infant memory during the triphasic period of sleep development. We used an elicited imitation task, in which infants learned new objects and actions before both their morning and afternoon naptimes. In the Nap-Nap condition, infants' memory was probed following both naps. In the Wake-Nap condition (within subjects), infants were kept awake for their morning nap but engaged in an unrestricted afternoon nap, with memory tested across the morning wake period and afternoon nap. We specifically manipulated the morning nap for two main reasons. First, the morning nap has been reported previously to "disappear" earlier developmentally, with children consolidating their naps into one midday or afternoon nap by 15-24 months (Weissbluth, 1995). Thus, the cognitive function of this nap may be more unique at earlier ages than that of the midday or afternoon nap, the utility of which has already been examined in studies of biphasic children (e.g., Kurdziel et al., 2013; Lokhandwala & Spencer, 2020). Second, depriving infants of the morning nap would allow us to observe how naps might interact with one another at this age (in particular, how deprivation affects the structure and function of the subsequent afternoon nap). In contrast, manipulating the afternoon nap would not necessarily have any effect on the prior morning nap, and thus would not provide insights into the dynamic relations between daytime sleep bouts in infancy. During each nap, we also measured infants' naps using polysomnography (PSG), the gold standard for capturing sleep staging and microstructure.

We hypothesized that taking both naps would benefit memory performance. Specifically, we expected that infants' morning memory performance would decline when kept awake during the morning nap, and that the detrimental effect of staying awake could also carry over into the afternoon due to the effects of fatigue on later encoding (which also subsequently impacts nap-dependent memory changes; Stickgold, 2009). However, we also hypothesized that individual differences in infant nap physiology might influence both the extent to which the morning nap is beneficial (i.e., with lighter, less SWA-rich naps being less beneficial), and the extent to which the afternoon nap can aid memory across different morning conditions. We hypothesized that morning and afternoon naps would both include high amounts of N2 sleep and SWS, given previous studies of single naps in this age range (the average timing of which varied between morning and afternoon in different studies) and across early childhood (e.g. Horváth, Hannon, Ujma, Gombos, & Plunkett, 2018; Friedrich et al., 2020, 2017; also see Mantua & Spencer, 2017 for review). On the other hand, considering

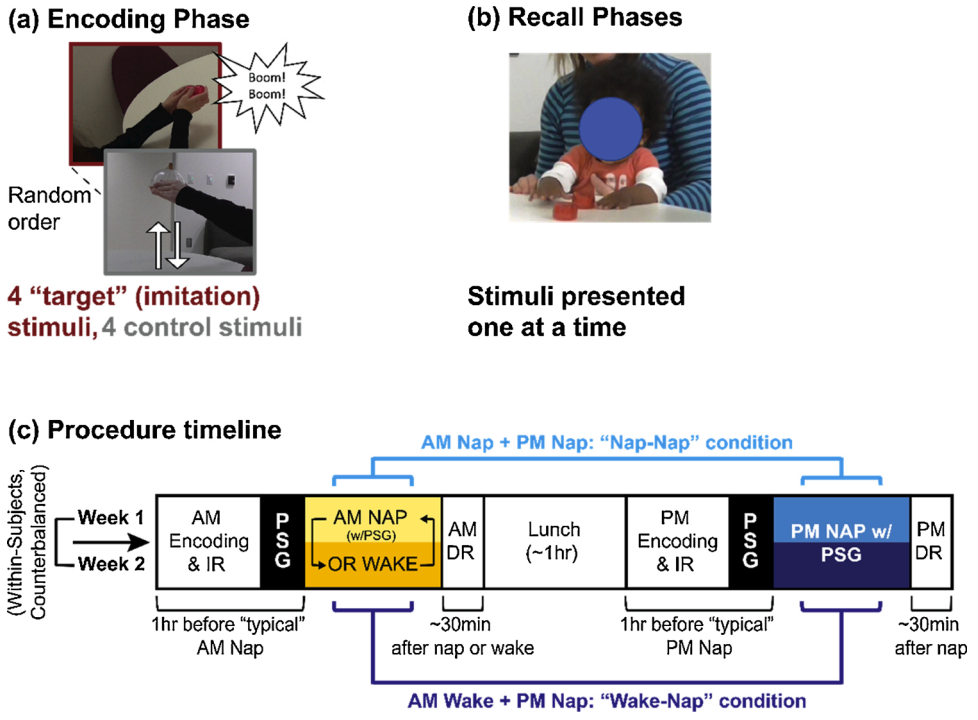

Fig. 1. Task and study design. (a) During encoding, infants watched an experimenter manipulate 8 objects. For 4 objects (targets), the experimenter produced effect-producing (e.g., noisemaking) "target" actions, whereas for the other four (control), the experimenter lifted the object and set it down. The experimenter demonstrated actions 3x per object, with object presentation order randomized. (b) For recall, infants were given each object to manipulate for approximately 30 seconds each. (c) Study timeline. IR = Immediate recall phase; PSG = Polysomnography; DR = Delayed recall phase; AM = Morning; PM = Afternoon. The two study conditions ("Nap-Nap" and "Wake-Nap") were within-subjects, with condition order counterbalanced and occurring approximately 1-2 weeks apart.

the physiological differences in morning and afternoon naps previously reported in adults (Groeger et al., 2011; Karacan et al., 1975), as well as the finding in young children that nap SWS and SWA may vary as a function of age and time spent awake (Kurth, Lassonde et al., 2016), an alternative hypothesis is that afternoon naps may contain more SWS and SWA than morning naps. Furthermore, we hypothesized that accumulation of sleep pressure in the Wake-Nap condition would lead to increased SWA in the afternoon nap relative to the afternoon nap in the Nap-Nap condition (Borbély, Baumann, Brandeis, Strauch, & Lehmann, 1981; Brunner, Dijk, Tobler, & Borbély, 1990; Knoblauch, Kräuchi, Renz, Wirz-Justice, & Cajochen, 2002).

No prior research has examined correlations between the task examined here (object-action imitation) and infants' sleep architecture or microstructure. However, based on infant and child studies using other declarative tasks, and on previously proposed sleep mechanisms (2020, Diekelmann & Born, 2010; Friedrich et al., 2019; Kurdziel et al., 2013; Lokhandwala & Spencer, 2020; Simon et al., 2017), we hypothesized that slow waves and sleep spindles would be involved, and that time spent in the sleep stages in which these events occur (N2 and SWS) might also help to predict the depth of memory retention and consolidation occurring across nap intervals. Overall, we anticipated that our study would provide an initial exploration into the structure and memory implications of triphasic sleep during infancy.

2. Methods

2.1. Study Design

We used a within-subjects design, with infants completing two separate study conditions in our sleep laboratory (Nap-Nap and Wake-Nap, as described above). Conditions were scheduled 1-2 weeks apart, and condition order and stimulus sets were counterbalanced across participants. More information on the timeline and procedures for each condition is in Fig. 1 and **Procedures** (Section 2.4).

2.2. Participants

Fifteen 9-month-old infants (5 female, mean age at session 2 = 9 mos, 22 days, range 9 mos 12 days – 10 mos 7 days) and their caregivers completed the current study. The age range of 9 months was specifically chosen because it is the age at which 2 naps per day is the most common sleeping schedule according to prior longitudinal studies and meta-analyses (Galland et al., 2012; Weissbluth, 1995). Families were initially recruited through community/online flyers and advertisements and an opt-in family contact database managed across laboratories. Infant eligibility criteria included: no known vision impairments; no known diagnoses of developmental, learning, sleep, or neurological disorders; and normal gestation length and birthweight (note that one child on the "borderline" of premature (gestation <37 weeks) was still included in the present study as her birthweight was in the normal range). All infants were also required to be napping twice per day on average (according to caregiver's report) at the time of recruitment. Of the 15 infants, 12 were identified by their caregivers as White; 2 were Biracial (White and Chinese, and White and Pacific Islander); and one caregiver did not respond. Additionally, 2 infants were identified as being of Hispanic/Latinx origin. All caregivers completed at least some college (3 had not yet completed a degree; all others had obtained a Bachelor's degree or higher), and the median household income for the sample (uncorrected for number of household members) was in the range of $40,001-$70,000.

Of the 15 participants in the study, all infants contributed some behavioral data, whereas specific sleep physiology measures depended on PSG data quality. Specifically, one infant who completed the study had a skin condition that prevented PSG recording; thus, this infant was excluded from all PSG analyses. Additionally, one caregiver felt uncomfortable including the PSG for their child's afternoon nap in the Wake-Nap condition (see procedure below), while another infant's morning nap was unscorable due to excessive EEG noise and intermittent loss of mastoid references. Thus, for calculating sleep stage distributions, 13 infants contributed scorable morning nap PSG; 14 contributed afternoon nap PSG in the Nap-Nap condition; and 13 contributed afternoon nap PSG in the Wake-Nap condition. Finally, for sleep microstructure analyses (across-hemisphere spindle densities and SWA), only 12 morning naps, 12 afternoon naps in the Nap-Nap condition, and 11 afternoon naps in the Wake-Nap condition could be included, due to loss of one mastoid channel in the other PSG-recorded naps.

2.3. Materials/Apparatus

2.3.1. Imitation Task

To assess infants' memory, we used an elicited imitation task informed by previous paradigms in this age range (Bauer et al., 2000; Seehagen et al., 2015). Specifically, eight sets of four objects were constructed either by hand using infant-safe materials or from combining existing infant-safe objects from different sources (e.g., a plastic measuring spoon and a small steel bucket). Images and details, including scoring criteria, for each object are in Supplemental Table S1. For each Encoding phase of the task, two sets of objects were used (Fig. 1a). One set served as the "target" stimuli, in which the experimenter performed specific target actions for each object, while the other set served as "control" stimuli, in which the experimenter simply lifted the objects up and down. Control stimuli served as a manipulation check to assess infants' spontaneous production of target actions (Supplemental Table S2). Object presentation order was randomized for each participant, and target and control sets were counterbalanced across participants and across morning/afternoon assessments. Additionally, the same experimenter performed the imitation task for all infants.

Infant behavior during the task was recorded with a Sony® HandyCam HDR camcorder in a tripod camera holder. A standard circular table was used to present the stimuli, with chairs high enough for the infant (held by their caregiver) to manipulate the objects

on their own when placed in front of them.

2.3.2. Polysomnography (PSG)

Infant nap physiology was recorded using a 32-channel PSG system (EasyCap; Brain Products GmbH) with a sampling rate of 500 Hz in combination with a compact bluetooth-compatible amplifier (LiveAmp; Brainvision, Brain Products GmbH). The electrode montage included 24 EEG electrodes (Fz, F3, F4, F7, F8, FCz, FC1, FC2, FC5, FC6, C3, C4, CP1, CP2, CP5, CP6, Pz, P3, P4, P7, P8, POz, O1, and O2), two electrooculography electrodes, and two electromyography electrodes affixed to the chin area and referenced to one another. EEG channels were recorded relative to a ground electrode at position FPz, and were referenced to an electrode at position Cz along with the contralateral mastoids at positions A1 and A2.

2.3.3. Additional sleep and background measures

Infant habitual sleep was recorded with an actigraph monitor (Actiwatch Spectrum, Philips Respironics, Bend OR) placed on the ankle (Sadeh, Acebo, Seifer, Aytur, & Carskadon, 1995) for 1-2 weeks between testing days. The actigraph collected movement data in 15-second epochs, with a sampling frequency of 32 Hz and a sensitivity of <0.01 g. Caregivers completed a daily sleep diary between testing days, and completed the Brief Infant Sleep Questionnaire (BISQ; Sadeh, 2004) assessing infants' sleep habits. An in-house health and demographic questionnaire was also administered.

2.4. Procedure

All materials and procedures were approved by the Institutional Review Board of the University of Massachusetts, Amherst, and all work was carried out in accordance with the Declaration of Helsinki for experiments involving human participants. Prior to participation, all caregivers provided written informed consent, and infants' behavior was monitored for implied assent throughout the study sessions.

General procedures and timeline for the study are summarized in Fig. 1. For both study conditions (Nap-Nap and Wake-Nap), caregivers were asked to arrive at the sleep lab approximately 1 hour before their child's typical morning nap time. After caregivers filled out informed consent and allowed infants to become acquainted with the surroundings, experimenters began the morning *Encoding phase* (Fig. 1a and c), in which 8 objects (4 target, 4 control) were presented to the infant by an experimenter seated across the table. During encoding, the experimenter presented each object action three times (Konrad et al., 2018; Seehagen et al., 2015) before removing the object from view and introducing the next object.

Following encoding, infants had a 5-10 minute break prior to the *Immediate recall* phase (Fig. 1b–c) in which infants were handed the objects one at a time and the experimenter asked, "What do we do with this?" Caregivers were instructed not to interfere or provide any prompting to the infant, and infants were faced away from their caregiver while sitting on the caregiver's lap at the table. Infants were then given approximately 30 seconds (paused intermittently if the infant dropped, threw, or pushed the object out of reach) to manipulate each object. Following the Immediate recall phase, infants took another short break for feeding and diapering, and were then outfitted with PSG before either being nap-promoted according to their typical routine (Nap-Nap condition) or kept awake (Wake-Nap condition). Infants napped in a laboratory bedroom furnished with a crib and a baby monitor. A hook on the crib was used to mount the PSG amplifier out of reach of the infant. Infants were monitored visually through a two-way window and the baby monitor. In the Wake-Nap condition, infants remained in the laboratory, either playing quietly in the living room area with lab-provided toys or pushed in their stroller. The morning wake interval was meant to be as long at the infants' typical morning nap duration (as reported by the caregiver), or 1 hour in length at minimum. Approximately 30 minutes following the nap or wake interval, infants completed the *Delayed recall phase*, in which an experimenter again handed infants the previous objects and allowed them to manipulate each for 30 seconds. Following the morning Delayed recall, infants and caregivers were permitted to leave the laboratory or remain in the laboratory area for lunch. In the afternoon, infants returned to the laboratory approximately an hour before their afternoon naptime and encoded a new set of 8 objects (Fig. 1c). Infants' Immediate recall for the new objects was then tested using the same procedures as in the morning Immediate recall phase. PSG was reapplied followed by afternoon nap promotion for both conditions. Infants' Delayed recall was tested for the new objects approximately 30 minutes after waking from their nap. All components of the memory tasks (Encoding, Immediate recall, and Delayed recall) were video-recorded.

2.5. Infant Memory Scoring

To assess infants' memory for experimenters' target actions, four coders blind to which sets of objects were the "targets" for each infant reviewed and scored infants' behaviors with each object during the Immediate and Delayed recall phases. Scoring was completed using ELAN Eudico Linguistic Annotation software version 5.5 (https://archive.mpi.nl/tla/elan; Sloetjes & Wittenburg, 2008). Two coders scored ~54% of all videos, and also scored an overlapping subset of 87.5% of one another's videos for interrater reliability analyses. The other two coders scored the remaining ~46% of all videos, including an overlapping subset of 60% of one another's videos for reliability analyses. Cronbach's α and absolute intraclass correlation coefficients (two-way mixed, single-measures) for infants' overall recall scores across phases were in the good to excellent ranges for both pairs of coders (Pair 1: $\alpha = .92$, ICC = .83; Pair 2: $\alpha = .89$, ICC = .78), suggesting good internal consistency and high absolute interrater reliability.

2.6. Sleep Scoring and Microstructure Quantification

All sleep recordings were band-pass filtered using cutoffs of 0.3-35 Hz for the EEG/EOG channels, and 10-70 Hz (with a 60 Hz Notch filter) for the muscle channels. Channels were then re-referenced to the contralateral mastoid (previously referenced to Cz during recording), and sleep stages were visually scored in RemLogic version 3.4.1 (Embla® Systems, Natus Medical) in 30-sec epochs using pediatric criteria (Iber, Ancoli-Israel, Chesson, & Quan, 2007).

Following manual sleep scoring, nap microstructure (spindles and SWA) was assessed in MATLAB using the toolboxes EEGLAB (Delorme & Makeig, 2004) and ERPLAB (Lopez-Calderon & Luck, 2014) in combination with custom software (PSGpower; Jones, Fitzroy, & Spencer, 2019; Lokhandwala & Spencer, 2020). To identify sleep spindles across non-REM (N2 and SWS), we used an autodetection algorithm previously described by Ferrarelli et al. (2007), and applied Mcclain et al. (2016)'s upper and lower detection threshold values established for child populations. For the autodetection algorithm, the unfiltered PSG recordings were re-referenced to the averaged mastoid and bandpass filtered with a Chebyshev Type II minimum-order filter, using passband frequencies of 11 and 15 Hz and stopband frequencies of 10 and 16 Hz. The rectified signals for each electrode of interest were then converted to amplitude envelopes by extracting their peaks. In line with Mcclain et al. (2016), and to allow for participant-specific amplitude criteria, spindles were identified when the envelopes exceeded an upper threshold of six times the average envelope amplitude. Once spindles were identified, onset and offset times for each spindle were identified at the closest preceding and following times relative to the spindle peak in which the envelope amplitude fell below a lower threshold of two times the average amplitude. Spindle densities were then calculated by dividing the total number of spindles detected during non-REM sleep by the total duration (in minutes) of non-REM sleep. Given prior work suggesting that frontal and centroparietal spindles may differ in their influence on declarative memory in infants (Friedrich et al., 2020; Friedrich et al., 2019), we calculated average spindle densities separately for frontal spindles (averaging the densities across channels F3 and F4) and centroparietal spindles (averaging the densities across channels C3, C4, P3, and P4).

To extract SWA, we used the Hilbert envelope transformation method as described previously in Jones et al. (2019) and Lokhandwala and Spencer (2020). Briefly, the raw PSG data were again re-referenced to the averaged mastoid, and bandpass filtered into delta activity (0.5-4 Hz) using a Butterworth infinite-impulse response filter (order 2). Using the Hilbert transformation (see Jones et al., 2019 for further details), delta amplitude envelopes were then extracted for each electrode of interest, and normalized by total time in SWS. From this procedure, SWA was defined as delta density, i.e., the average absolute value (magnitude) of the artifact-free delta amplitude envelope summed per second of SWS. In line with prior infant findings (Simon et al., 2017), we focused on fronto-central SWA, and thus averaged the summed per-second amplitude envelope values across channels F3, F4, C3, and C4.

For one infant, channel F4 was faulty for all naps. Analyses were run with F4 excluded and again with F4 replaced by F8. All significant findings remained using either method; thus, findings reported here are those with F4 excluded.

2.7. Statistical Analyses

All primary statistical analyses were performed in SPSS version 25. To probe the effects of different and multiple naps on infant memory, we first ran two repeated-measures ANOVA analyses including the factors of Nap Interval (AM vs. PM) and Recall Phase (Immediate vs. Delayed) on infants' memory scores, running separate ANOVAs for the Nap-Nap and Wake-Nap conditions. Infants' memory scores were calculated as the percentage of total imitation points earned for target objects out of the total possible within each recall phase, and significant ANOVA effects were subsequently followed up with planned comparisons t-tests assessing specific contrasts of interest to our study hypotheses. We also used repeated-measures ANOVAs to examine differences in the physiological characteristics (stage distributions, spindle densities, and SWA) of infant naps. Sphericity assumptions were checked using Mauchly's test of sphericity, and violations were accounted for by applying the Greenhouse-Geisser correction.

We used Pearson's correlations (or Spearman's, when variables deviated from normality according to a significant Shapiro-Wilk test and subsequent inspection of skewness and kurtosis) to investigate how specific sleep characteristics (time in N2 and SWS, SWA, and spindle densities) correlated with changes in memory across the corresponding nap interval. For these analyses, change in memory was calculated by subtracting the Immediate recall score from the Delayed recall score for each nap, meaning that positive values indicate memory improvement over the nap.

3. Results

3.1. Memory Performance

To assess the relative benefits of morning and afternoon naps on infants' memory retention (Fig. 2a–b), we first compared differences in memory retention—i.e., immediate (pre-nap) vs. delayed (post-nap) recall—between the morning and afternoon naps in the Nap-Nap condition (i.e., the condition in which infants napped both in the morning and afternoon). More specifically, we ran a 2 × 2 repeated-measures ANOVA including the predictors of nap interval (morning vs. afternoon) and recall phase (immediate vs. delayed), with infant memory scores as the dependent variable. There was no main effect of nap interval ($F(1,14) = .15$, $p = .71$, $\eta_p^2 = .01$), suggesting that memory performance was similar overall between morning and afternoon naps in this condition (Fig. 2a, b). There was also no significant main effect of recall phase ($F(1,14) = 1.62$, $p = .22$, $\eta_p^2 = .10$), indicating that infants' memories were relatively preserved from immediate to delayed testing when collapsed across naps. Furthermore, the interaction between nap interval and recall phase was not significant ($F(1,14) = .10$, $p = .76$, $\eta_p^2 = .007$), suggesting that the extent of infants' memory change from immediate to delayed testing did not differ by nap.

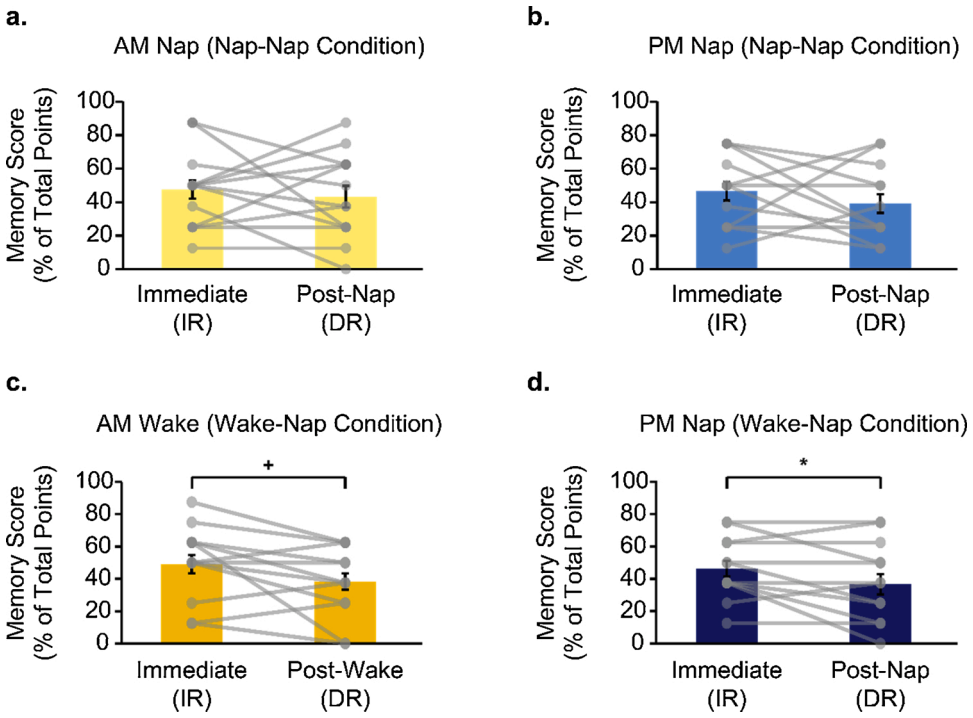

Fig. 2. Nine-month-olds' memory performance (calculated as a percentage of imitation points earned for target objects out of the total possible) before and after their nap and wake intervals during the experimental sessions. Panels (a) and (b) depict infants' memory performance across their morning nap (a) and afternoon nap (b) in the Nap-Nap condition, while panels (c) and (d) depict infants' performance across their morning wake interval (c) and afternoon nap (d) in the Wake-Nap condition. Error bars on bar plots represent ±1SE, while grey translucent lines overlaying the plots illustrate performance changes for individual infants. IR = Immediate Recall Phase; DR = Delayed Recall Phase. + p = .054; *p = .035.

Next, to explore how staying awake in the morning impacted infants' memory scores across the morning wake interval and subsequent afternoon nap (Fig. 2c–d), we ran a 2 × 2 repeated-measures ANOVA including the factors of interval (morning vs. afternoon) and recall phase (Immediate vs. Delayed) as predictors of infants' memory scores in the Wake-Nap condition. Similar to the Nap-Nap condition, we found no significant main effect of interval ($F(1,13^{1}) = .071$, $p = .79$, $\eta_p^2 = .005$), suggesting that overall memory performance did not differ significantly between the morning wake interval and the afternoon nap interval. However, there was a significant main effect of recall phase ($F(1,13) = 6.36$, $p = .025$, $\eta_p^2 = .33$), with infants' delayed performance being significantly lower than their immediate recall when collapsed across the AM and PM periods. The interaction between nap interval and recall phase was not significant, $F(1,13) = .027$, $p = .87$, $\eta_p^2 = .002$.

To probe further whether our significant main effect of recall phase in predicting infants' memory scores in the Wake-Nap condition was driven more by changes in recall across the morning wake period than by changes across the afternoon nap period, we conducted two planned within-subjects t-tests: one assessing Immediate vs. Delayed recall scores across the morning wake interval, and the other assessing Immediate vs. Delayed recall scores across the afternoon nap interval in the Wake-Nap condition. Consistent with our predictions, there was a decrease in memory scores across the morning wake interval; however, this difference was marginal ($t(14) = 2.10$, $p = .054$, $d = .54$; Fig. 2c). In contrast, the decrease in memory scores across the afternoon nap interval was significant ($t(13) = 2.35$, $p = .035$, $d = .63$; Fig. 2d). We also ran an exploratory t-test assessing whether encoding differed for morning and afternoon intervals in the Wake-Nap condition, with the reasoning that staying awake in the morning may have negatively affected afternoon encoding. The difference in encoding scores was not significant between the two intervals ($t(13) = .30$, $p = .77$, $d = .08$).

3.2. Sleep Structure (Physiology) of AM and PM Naps

Our next aim was to investigate whether infants' nap bouts differed in their physiological characteristics, including architecture

[1] For this condition only, one infant was excluded due to a camera angle issue in which the infants' actions during afternoon testing were obscured from view by the caregiver's arms, which she placed on the table to discourage the infant from throwing objects on the floor.

Table 1
Sleep Characteristics of Polysomnography-Recorded Infant Naps (n = 12[a])

Sleep Variable	AM Nap Mean (SD)	PM Nap (Nap-Nap condition) Mean (SD)	PM Nap (Wake-Nap condition) Mean (SD)
Sleep onset time (hr:min)	10:06 (±46 min)	14:15 (±62 min)	12:49 (±47 min)
Total sleep time (TST; min)	77.83 (±36.06)	90.08 (±33.97)	69.04 (±28.55)
Time in bed from sleep onset to final wake (min)	82.71 (±37.98)	96.21 (±34.75)	71.42 (±28.17)
% Wake after sleep onset (WASO)	5.61 (±4.49)	6.40 (±6.27)	3.47 (±6.35)
% Non-REM stage 1 (N1)	12.13 (±7.91)	8.29 (±6.50)	4.13 (±4.31)
% Non-REM stage 2 (N2	39.36 (±16.41)	36.78 (±18.51)	37.05 (±21.87)
% Non-REM stage 3 (SWS)	41.03 (±20.40)	42.63 (±21.03)	51.52 (±20.42)
% REM	1.87 (±3.63)	5.90 (±10.34)	3.82 (±8.27)
Frontal spindle density,	1.68 (±1.73)	1.60 (±1.34)	1.77 (±1.23)
Centroparietal spindle density,	1.40 (±1.71)	1.46 (±1.36)	1.68 (±1.32)
Slow-wave activity (SWA)[b]	19056.98 (±3524.71)	19663.80 (±3556.98)	20413.41 (±3101.23)

[a] For these descriptives, only infants with measures for all 3 naps are included.
[b] Slow-wave activity is captured in arbitrary amplitude envelope units summed per second of slow-wave sleep. These units can be converted into mean amplitude envelope units (analogous to microvolts) by dividing by our EEG sampling rate of 500 Hz. For more information on the Hilbert envelope method applied here, please refer to Jones et al., 2019.

and microstructure (Table 1). To explore whether the overall percentage distribution of sleep stages (architecture) differed for infants' morning and afternoon naps, we ran a 4 × 2 repeated-measures ANOVA including sleep stage (4 levels[2] : N1, N2, SWS, and REM) and nap interval (2 levels: morning vs. afternoon in the Nap-Nap condition) as predictors of infants' percent of sleep time, comparing the morning and afternoon naps from the Nap-Nap condition. Unsurprisingly, there was a significant main effect of sleep stage on infants' percent of sleep time (Greenhouse-Geisser: $F(1.25,15.05) = 22.62$, $p < .001$, $\eta_p^2 = .65$). Follow-up Bonferroni tests indicated that infants spent significantly less time in REM sleep than in all other stages, including N1 (mean difference = 6.18%, $p = .032$; *ps* comparing REM with N2 or SWS < .001), and that infants also spent less time in N1 than in stages N2 or SWS (both *ps*<.003; Table 1). In contrast, the difference in time spent in N2 vs. SWS was not significant (p > .99). There was also no significant main effect of nap interval ($F(1,12) = .56$, $p = .47$, $\eta_p^2 = .04$), indicating that average combined percentage of time asleep during each nap (as opposed to awake, which was the reference level in this analysis[2]) did not differ between the morning and afternoon naps. Additionally, the interaction between sleep stage and nap interval was not significant (Greenhouse-Geisser: $F(1.65,19.77) = 1.26$, $p = .30$, $\eta_p^2 = .10$), indicating no significant differences in percentage distribution of specific sleep stages (N1, N2, SWS or REM) between the morning and afternoon naps.

We also examined how morning nap deprivation affected infants' afternoon nap architecture. We ran another 4 × 2 repeated-measures ANOVA evaluating the effects of sleep stage (4 levels[2]) and nap interval (2 levels: afternoon nap in Nap-Nap condition vs. afternoon nap in Wake-Nap condition) on infants' percent sleep time in the afternoon naps from the Nap-Nap vs. Wake-Nap conditions. Again (and unsurprisingly), we found a significant main effect of sleep stage on infants' sleep time (Greenhouse-Geisser: $F(1.16,13.95) = 28.62$, $p < .001$, $\eta_p^2 = .71$). Similar to the previous nap comparison, follow-up Bonferroni tests indicated that infants spent less time in REM than in N2 or SWS, and less time in N1 than in N2 or SWS (all *ps*<.001), with no difference in relative sleep time spent in N2 vs. SWS ($p > .99$). However, unlike in the ANOVA comparing morning and afternoon naps, there was no significant difference in the percentage of time spent in N1 vs. REM when collapsed across afternoon naps (mean difference= 1.7%, $p > .99$), indicating perhaps that the difference between N1 and REM in the prior analysis was driven by higher amounts of N1 in the morning nap (Table 1). Regarding the other effects, we again found no significant main effect of nap interval on infants' percent sleep time ($F(1,12) = 1.26$, $p = .28$, $\eta_p^2 = .10$), and no significant sleep stage x nap interval interaction (Greenhouse-Geisser: $F(1.78,21.36) = .52$, $p = .58$, $\eta_p^2 = .04$), indicating no significant differences in percentage distribution of specific sleep stages (N1, N2, SWS or REM) between the two afternoon naps.

Next, we assessed whether sleep microstructure—specifically, spindle densities and SWA—differed across infants' naps. To examine spindle densities across morning and afternoon naps, we ran a 2 × 2 ANOVA including nap interval (morning vs. afternoon in the Nap-Nap condition) and spindle type (frontal vs. centroparietal derivations) as predictors of infants' spindle densities during non-REM sleep (N2 and SWS combined). There were no main effects of either nap interval or spindle type on infants' spindle densities (Nap interval: $F(1,10) = .88$, $p = .37$, $\eta_p^2 = .080$; Spindle type: $F(1,10) = 2.27$, $p = .16$, $\eta_p^2 = .19$), nor was there a nap x spindle type interaction ($F(1,10) = .018$, $p = .90$, $\eta_p^2 = .002$). We also ran a 2 × 2 ANOVA comparing spindle type for afternoon naps in the Nap-Nap vs. Wake-Nap condition. Again, we found no main effects of either nap or spindle type on infants' spindle densities during non-REM (both ps>.30), nor a nap x spindle type interaction ($F(1,10) = 1.44$, $p = .26$, $\eta_p^2 = .13$). Together, these results suggest that both frontal

[2] Percentage of WASO was excluded from the ANOVA because it allowed for nap interval to be added as a factor whose main effect can be compared (as otherwise, the stages across both naps would sum to 100%, and have the same average, when all stages were collapsed together). However, comparison of WASO percentages can be gleaned from the main effect of nap interval in the analysis. For a similar analysis strategy applied to percent compositions, please refer to Deák et al., 2014; Mason, Kirkpatrick, Schwade, & Goldstein, 2019.

and centroparietal channel-derived spindle densities do not significantly differ between naps.

To evaluate differences in SWA across naps, we ran two within-subjects t-tests: one comparing SWA between the morning and afternoon naps in the Nap-Nap condition, and another comparing SWA between the afternoon naps taken under the Nap-Nap vs. Wake-Nap condition. Neither t-test was significant (both *ps*>.22), despite afternoon naps in the Wake-Nap condition appearing to have slightly elevated SWA on average (as expected given the prior nap deprivation; Table 1).

3.3. Relations between Infant Memory Change and Nap Physiology

In a preliminary analysis, we explored how infants' nap physiology (specifically, time in N2 and SWS, as well as spindle densities and SWA) predicted memory retention. We first examined whether time in N2 or SWS correlated with memory change scores across any nap as in previous developmental literature (Fig. 3). For both the morning and afternoon naps in the Nap-Nap condition, neither time in N2 nor SWS correlated with infants' memory change across the corresponding nap (all ps>.14; Fig. 3a–b). In contrast, for the afternoon nap taken in the Wake-Nap condition, we found a significant positive correlation between the total time spent in N2 (but not SWS) and memory change over the nap ($r = .62$, $p = .032$; Fig. 3c). To ensure that this correlation did not reflect difference in nap length, we re-ran this as a partial correlation controlling for infants' total nap length. The correlation became marginal ($r = .54$, $p = .085$), suggesting that this effect was perhaps partly, but not entirely accounted for by longer nap length.

Next, we probed whether infants' nap microstructure (spindles and SWA) correlated with changes in memory across each nap. With respect to spindle densities across non-REM (Appendix ASupplemental Figure S1), we found no significant correlations between spindle densities in either the frontal or centroparietal derivations and memory change across any nap (all ps>.103). However, we found associations between nap SWA and change in memory (Fig. 4). Specifically, in the Nap-Nap condition, higher SWA during the afternoon nap was marginally positively correlated with memory change over that nap ($r = .53$, $p = .075$; Fig. 4b). In contrast, for the afternoon nap in the Wake-Nap condition, higher SWA during this nap was significantly *negatively* correlated with memory change ($r = -.66$, $p = .026$; Fig. 4c). The correlation between SWA and memory change across the morning nap in the Nap-Nap condition was not significant ($r = .24$, $p = .46$; Fig. 4a).

4. Discussion

In this study, we investigated the features and benefits of infants' multiple nap bouts at 9 months, an age during which triphasic

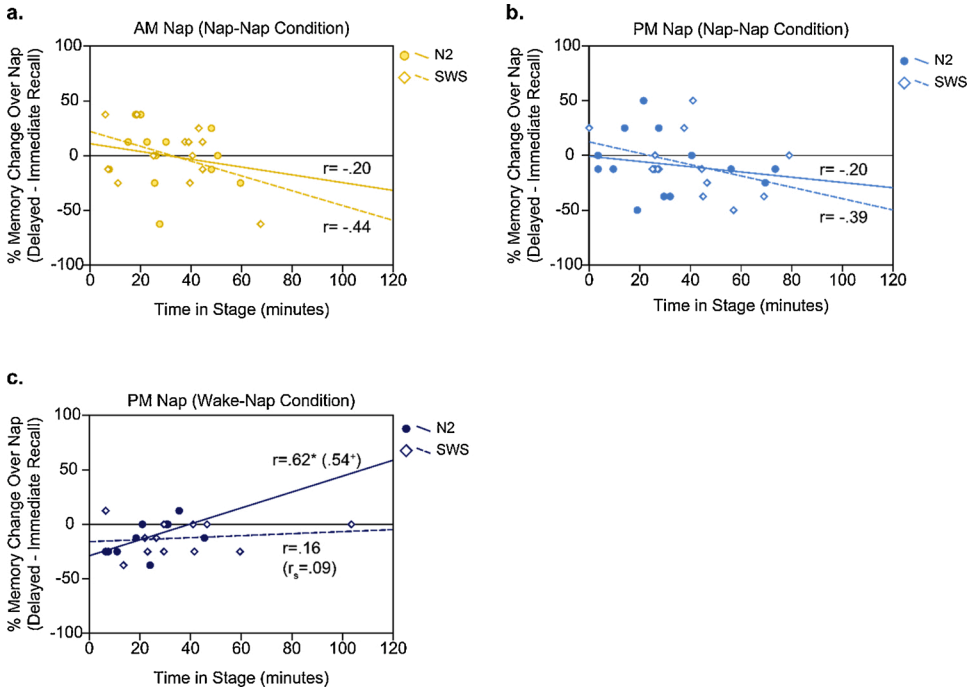

Fig. 3. Relations between infants' memory change across napping, and time spent in N2 and SWS. **a**) Depicts relations between memory change across the morning nap in the Nap-Nap condition and infant N2/SWS in the morning nap, while **b**) shows relations between memory change across the afternoon nap in the Nap-Nap condition and afternoon N2/SWS. **c**) Illustrates relations between memory change across the afternoon nap in the Wake-Nap condition, and infant N2/SWS in that nap. In **c**), *p = .032; +p = .085 when controlling for total nap duration overall; additionally, both Pearson's r and Spearman's rho are reported for the SWS correlation, as the distribution of time in SWS for this nap was slightly nonnormal according to kurtosis measures.

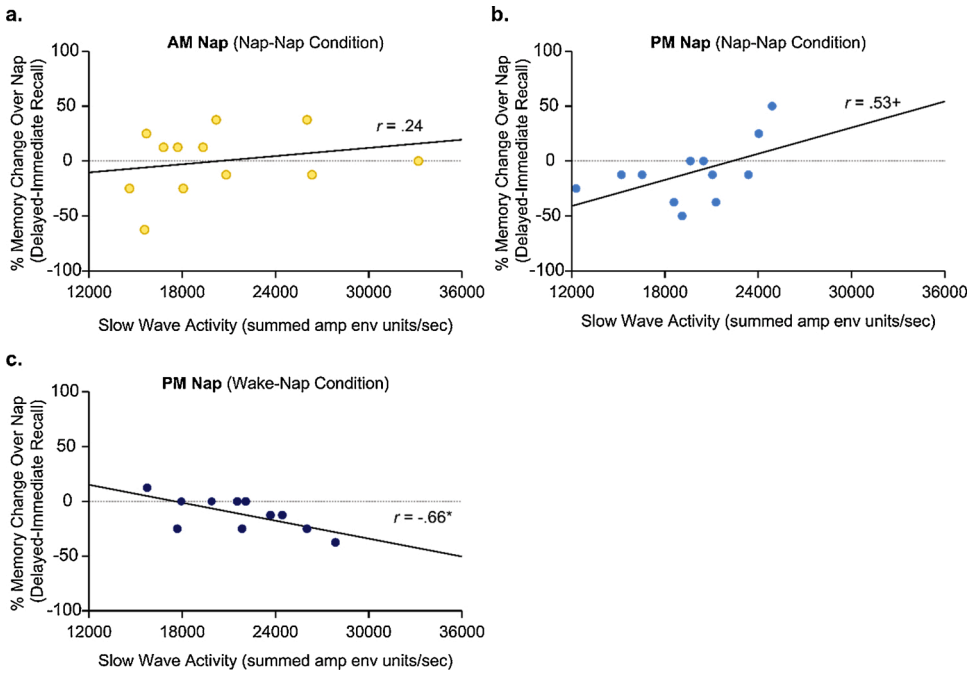

Fig. 4. Relations between infants' memory change across napping, and slow-wave activity (SWA) during slow-wave sleep (SWS). **a**) Depicts relations between memory change across the morning nap in the Nap-Nap condition and infant SWA in the AM nap, while **b**) shows relations between memory change across the afternoon nap in the Nap-Nap condition and afternoon SWA. **c**) Illustrates relations between memory change across the afternoon nap in the Wake-Nap condition, and SWA in that nap. + p = .075; *p = .026.

sleep is common. Specifically, we assessed whether engaging in multiple naps per day at this age was beneficial for memories of objects and actions, as well as whether nap physiology (stage distributions and microstructure) and its relations to memory differed across naps (morning, afternoon, and afternoon following morning nap restriction). Broadly, our findings provide tentative support for the hypothesis that morning nap deprivation at 9 months is detrimental for infants' memory retention, particularly compromising the benefit of the afternoon nap. Furthermore, although the stage distributions and microstructural characteristics of infants' naps did not significantly differ across naps and conditions, we observed tentative differences in how such characteristics related to infants' memory performance across different nap intervals. Namely, while nap SWA did not predict infants' memory across morning naps, afternoon SWA correlated positively—albeit marginally—with post-afternoon memory when infants had also taken a morning nap. In contrast, SWA significantly negatively predicted across-nap afternoon memory when infants were deprived of their morning nap, perhaps due to differences in the relative contributions of "state" vs. "trait"-based factors driving SWA values across the two nap conditions (discussed further below).

Regarding our behavioral findings, we found a main effect of recall phase (immediate vs. delayed) on infants' memory in the Wake-Nap condition alone, indicating that on average, infants' memory declined across both test intervals (morning wake and afternoon nap) in that condition. Intriguingly, when we followed up our Wake-Nap main effect with tests of memory decline across the morning and afternoon periods separately, we were surprised to find that infants' memory decline was only significant for their afternoon memory performance. That is, infants' memory decay in the Wake-Nap condition was only significant for items learned in the afternoon, following an unrestricted afternoon nap. Although we had expected memory decay to be evident directly after the morning wake period (as has been observed in 3-5-yr-olds, i.e., Kurdziel et al., 2013; Lokhandwala & Spencer, 2020), we found that memory decay after morning wake was only marginally significant relative to infants' immediate recall. Taken together, these findings suggest that skipping a morning nap, while possibly being modestly detrimental to morning learning in infants, may disrupt the afternoon nap's ability to protect and consolidate memories learned later in the day. How it does so remains an open question, given that 1) morning wake did not seem to influence initial afternoon encoding levels and 2) that our analyses revealed no obvious differences in afternoon nap stage distribution or microstructure between the Nap-Nap and Wake-Nap conditions. However, one possible explanation is that morning wake resulted in greater interference for longer-term consolidation and retrieval of items learned in the afternoon, despite no disruption of immediate afternoon recall (Spencer, 2021). Additionally, it is possible that our measures of nap architecture and microstructure were not sensitive enough to pick up on important memory-relevant sleep changes brought about by morning nap deprivation, such as changes in the ratios of spindles coupled to slow oscillations (Solano, Riquelme, Perez-Chada, & Della-Maggiore, 2021) or changes in expression of hormones regulated by sleep and involved in memory and learning (e.g. cortisol; Tribble, Dmitrieva, Watamura, & LeBourgeois, 2015).

Prior work in adults has suggested that naps taken in the afternoon may have different sleep stage distributions than naps taken in the morning, including higher percentages of SWS in afternoon naps and (in some reports) more REM sleep in morning naps (Groeger

et al., 2011; Karacan et al., 1975). However, differences in morning and afternoon naps in triphasic infants, for whom this sleep pattern is habitual and who exhibit dramatically different sleep composition (Kurth, Lassonde et al., 2016; Mantua & Spencer, 2017; Ohayon, Carskadon, Guilleminault, & Vitiello, 2004), was unknown. There was no difference in REM across infants' morning and afternoon naps (Table 1). There were also no significant differences in non-REM sleep stages, though Stage 1 tended to be higher in morning naps. Furthermore, sleep microstructure (spindle densities and SWA) did not differ between morning and afternoon naps. Consistent with these similarities in architecture and microstructure, morning and afternoon naps taken in the Nap-Nap condition had similar overall benefits for memory, as the extent of memory retention and strengthening for morning and afternoon memories did not differ when both naps were taken.

Arguably, one of our more intriguing (though currently tentative) findings was that the nap physiological correlates of memory retention differed for afternoon naps across conditions. Specifically, even though average SWA itself did not differ significantly between afternoon naps as a function of morning wake, the relations between afternoon SWA and memory change over infants' afternoon naps appeared to depend on whether infants had also taken a morning nap. When infants took their morning nap, the correlation between afternoon nap SWA and afternoon memory changes trended in the positive direction; however, the opposite effect was found when infants were deprived of their morning nap, with afternoon SWA significantly negatively predicting memory performance. This condition-dependent dichotomy in SWA's relations to memory in the afternoon nap may reflect different factors driving SWA in these naps. For example, studies in both adults and children have shown that SWA, aside from possibly varying as a function of learning or other variables, increases with increasing time spent awake (Borbély et al., 1981; Kurth, Dean et al., 2016; Kurth, Lassonde et al., 2016). Though afternoon nap SWA in the Wake-Nap condition was not higher on average than afternoon nap SWA in the Nap-Nap condition, it is nonetheless possible that for individual infants, SWA in the Wake-Nap condition more closely reflects the extent of sleep pressure buildup from skipping the morning nap than it would under rested conditions. This may also explain why time in N2 was positively (though marginally, when controlling for total sleep time) correlated with memory only for the afternoon nap in the Wake-Nap condition, as time in N2 between infants in this condition may also reflect differences in sleep pressure accumulation for this nap.

In contrast, individual differences in afternoon nap SWA in the Nap-Nap condition may be less reflective of sleep pressure differences (given the prior morning nap opportunity), and more closely coupled to cortical maturational differences that may more robustly support sleep-dependent consolidation mechanisms (Diekelmann & Born, 2010). According to prior work, SWA increases across early development in parallel with cortical synaptic connectivity and density, reaching a peak shortly before puberty and declining thereafter (Feinberg & Campbell, 2013; Huber & Born, 2014; but see also Kurth, Lassonde et al., 2016). Given this trajectory of SWA change and brain development, it is reasonable to hypothesize that higher SWA in some infants compared to others would correspond to more robust memory consolidation under conditions of adequate prior rest. Although it is unclear why SWA during the morning nap did not relate to morning memory performance, it is possible that infants' overnight sleep prior to the morning nap may have influenced this association. As we did not experimentally manipulate overnight sleep in this study, it is difficult to determine the influence of sleep pressure accumulation vs. individual maturational differences on SWA in the morning nap as compared to the other naps. However, it is possible that some infants may not have gotten adequate overnight sleep at home prior to participating in the Nap condition, which could have in turn obscured the otherwise positive association between frontocentral SWA and memory across the morning nap. Nonetheless, when controlling for infants' average overnight sleep time using our background sleep measures (see supplemental analyses), the correlation between morning nap SWA and memory change over the morning nap remained nonsignificant. In general, our dichotomous findings across different nap periods may also speak to the discrepancies in the physiological correlates of nap-dependent memory consolidation observed across other studies in infancy (see Mason et al., 2021 for review), as prior studies have not always tightly controlled the specific nap period that they are examining even during ages in which their participants are presumably still triphasic.

One additional null finding is our lack of association between spindle densities and infant memory across napping. While we had hypothesis-driven reasons to examine spindle densities in particular in relation to memory (Friedrich et al., 2019; Kurdziel et al., 2013), it is important to note that various additional measures of spindles have also been examined and correlated with memory retention in child studies. Such measures include root-mean-squared (RMS) spindle amplitude (Friedrich et al., 2020) and power spectral density, among others (Friedrich et al., 2017). Furthermore, other work has drawn distinctions between the function of fast spindles (12-15 Hz) and slow spindles (9-12 Hz), as well as between spindles that are coupled to the up vs. down-states of co-occurring slow oscillations (Diekelmann & Born, 2010; Friedrich et al., 2020; Hahn et al., 2021; Mölle, Bergmann, Marshall, & Born, 2011; Staresina et al., 2015). As the relative coupling of spindles and slow oscillations may be critical for successful transfer of memories from hippocampus to longer-term cortical memory stores, it is possible that our analysis of spindle densities, which was limited to assessing the number of spindles per minute across N2 and SWS regardless of coupling, was not nuanced enough to truly capture the role of spindles in infants' nap-dependent declarative memory consolidation. Alternatively, consolidation of memories of specific objects and actions at this age may simply be less dependent on spindles and more dependent on SWA for reasons that are task- or memory type-specific. Regardless, though most sleep-dependent consolidation theories describe spindles and slow waves as working together within the same mechanism, it is not uncommon in developmental work to find differences in the strength of association between memory performance and these two features of sleep (see Mason et al., 2021 for review). These differences could be due to individual sample characteristics as well as small sample sizes, which are common in developmental work and (as noted below) a limitation of the present study.

Although our within-subjects design and thorough experimental measurement and control of infants' naps are strengths of our current study, we acknowledge limitations. Perhaps most obviously, our sample is small compared to samples used for other sleep-dependent memory studies in this age range (e.g., Friedrich et al., 2020; Horváth et al., 2018; Seehagen et al., 2015), and is

composed of infants from primarily White, college-educated families. Though our within-subjects design helps to ameliorate concerns about sample size to some extent, our findings must nonetheless be replicated with a larger, more diverse sample to be able to generalize and further confirm our findings. Additionally, various other analysis methods could be used to quantify the microstructural features of infants' naps (e.g., spindle-slow oscillation coupling, power density spectra, etc.), which may shed more light on which features are important for memory. Along similar lines, the influence of triphasic sleep on other forms of memory and learning (such as language/grammar generalization, motor learning, and emotional memory) could also be explored in future work, to help determine whether triphasic sleep benefits these forms of learning in ways that are unique or similar to that of declarative memory for objects and actions.

Overall, our study adds to prior literature examining the benefits of infant napping on memory (2017, Friedrich et al., 2020; Seehagen et al., 2015; Simon et al., 2017), and extends it by examining the differences between specific nap bouts along with the benefits of multiple naps as compared to one nap alone. Though further replication is needed, we hope that our work will provide a first step in illuminating the functional importance of multiple daytime nap bouts for memory and learning during the triphasic phase of infant development.

Author Statement

GM Mason: Conceptualization, methodology, formal analysis, investigation, data curation, writing (original draft), writing (review and editing), visualization, project administration. **LBF Kurdziel**: Conceptualization, methodology, validation, data curation, writing (review and editing). **RMC Spencer**: Conceptualization, methodology, resources, writing (review and editing), supervision, funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgement

We would like to thank all of the families who participated in our work. This research was funded by a research project grant from the National Heart, Lung, and Blood Institute (R01 HL111695) awarded to Rebecca M.C. Spencer.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.infbeh.2021.101647.

References

Barr, R., Dowden, A., & Hayne, H. (1996). Developmental changes in deferred imitation by 6- to 24-month-old infants. *Infant Behavior and Development, 19*(2), 159–170. https://doi.org/10.1016/S0163-6383(96)90015-6.

Bauer, P. J., Wenner, J. A., Dropik, P. L., & Wewerka, S. S. (2000). Parameters of remembering and forgetting in the transition from infancy to early childhood. *Monographs of the Society for Research in Child Development, 65*(4), 1–204. i–vi.

Borbély, A. A., Baumann, F., Brandeis, D., Strauch, I., & Lehmann, D. (1981). Sleep deprivation: Effect on sleep stages and EEG power density in man. *Electroencephalography and Clinical Neurophysiology, 51*, 483.

Born, J., & Wilhelm, I. (2012). System consolidation of memory during sleep. March 4 *Psychological Research, 76*, 192–203. https://doi.org/10.1007/s00426-011-0335-6.

Brunner, D. P., Dijk, D.-J., Tobler, I., & Borbély, A. A. (1990). Effect of partial sleep deprivation on sleep stages and EEG power spectra: evidence for non-REM and REM sleep homeostasis. *Electroencephalography and Clinical Neurophysiology, 75*, 492–499. https://doi.org/10.1016/0013-4694(90)90136-8.

Bulf, H., Johnson, S. P., & Valenza, E. (2011). Visual statistical learning in the newborn infant. *Cognition, 121*(1), 127–132. https://doi.org/10.1016/j.cognition.2011.06.010.

Deák, G. O., Krasno, A. M., Triesch, J., Lewis, J., Sepeta, L., Dea, G. O., … Sepeta, L. (2014). Watch the hands: Infants can learn to follow gaze by seeing adults manipulate objects. *Developmental Science, 17*(2), 270–281. https://doi.org/10.1111/desc.12122.

Delorme, A., & Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods, 134*(1), 9–21. https://doi.org/10.1016/j.jneumeth.2003.10.009.

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews Neuroscience, 11*(2), 114–126. https://doi.org/10.1038/nrn2762.

Feinberg, I., & Campbell, I. G. (2013). Longitudinal sleep EEG trajectories indicate complex patterns of adolescent brain maturation. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology, 304*(4), 296–303. https://doi.org/10.1152/ajpregu.00422.2012.

Ferrarelli, F., Huber, R., Peterson, M. J., Massimini, M., Murphy, M., Riedner, B. A., … Tononi, G. (2007). Reduced sleep spindle activity in schizophrenia patients. *American Journal of Psychiatry, 164*(3), 483–492. https://doi.org/10.1176/ajp.2007.164.3.483.

Friedrich, M., Mölle, M., Friederici, A. D., & Born, J. (2019). The reciprocal relation between sleep and memory in infancy: Memory-dependent adjustment of sleep spindles and spindle-dependent improvement of memories. *Developmental Science, 22*(2), e12743. https://doi.org/10.1111/desc.12743.

Friedrich, M., Mölle, M., Friederici, A. D., & Born, J. (2020). Sleep-dependent memory consolidation in infants protects new episodic memories from existing semantic memories. *Nature Communications, 11*(1). https://doi.org/10.1038/s41467-020-14850-8.

Friedrich, M., Wilhelm, I., Mölle, M., Born, J., & Friederici, A. D. (2017). The Sleeping Infant Brain Anticipates Development. *Current Biology, 27*(15), 2374–2380. https://doi.org/10.1016/j.cub.2017.06.070. e3.

Galland, B. C., Taylor, B. J., Elder, D. E., & Herbison, P. (2012). Normal sleep patterns in infants and children: A systematic review of observational studies. *Sleep Medicine Reviews, 16*(3), 213–222. https://doi.org/10.1016/j.smrv.2011.06.001.

Goldstein, M. H., Schwade, J. A., & Bornstein, M. H. (2009). The value of vocalizing: Five-month-old infants associate their own noncry vocalizations with responses from caregivers. *Child Development, 80*(3), 636–644. https://doi.org/10.1111/j.1467-8624.2009.01287.x.

Gómez, R. L., & Edgin, J. O. (2016). The extended trajectory of hippocampal development: Implications for early memory development and disorder. *Developmental Cognitive Neuroscience, 18*, 57–69. https://doi.org/10.1016/j.dcn.2015.08.009.

Groeger, J. A., Lo, J. C. Y., Burns, C. G., & Dijk, D. J. (2011). Effects of Sleep Inertia After Daytime Naps Vary With Executive Load and Time of Day. *Behavioral Neuroscience, 125*(2), 252–260. https://doi.org/10.1037/a0022692.

Hahn, M. A., Bothe, K., Heib, D. P. J., Schabus, M., Helfrich, R. F., & Hoedlmoser, K. (2021). Slow oscillation-spindle coupling strength predicts real-life gross-motor learning in adolescents and adults. *BioRxiv*, 2021.01.21.427606.

Hayne, H., Boniface, J., & Barr, R. (2000). The development of declarative memory in human infants: Age-related changes in deferred imitation. *Behavioral Neuroscience, 114*(1), 77–83. https://doi.org/10.1037/0735-7044.114.1.77.

Horváth, K., Hannon, B., Ujma, P. P., Gombos, F., & Plunkett, K. (2018). Memory in 3-month-old infants benefits from a short nap. *Developmental Science, 21*(3), e12587. https://doi.org/10.1111/desc.12587.

Huber, R., & Born, J. (2014). Sleep, synaptic connectivity, and hippocampal memory during early development. *Trends in Cognitive Sciences, 18*(3), 141–152. https://doi.org/10.1016/j.tics.2013.12.005.

Iber, C., Ancoli-Israel, S., Chesson, A. L., & Quan, S. F. (2007). *The AASM manual for the scoring of sleep and associated events: Rules, terminology, and technical specifications* (1st ed.). Westchester, IL: American Academy of Sleep Medicine.

Jones, B. J., Fitzroy, A. B., & Spencer, R. M. C. C. (2019). Emotional memory moderates the relationship between sigma activity and sleep-related improvement in affect. *Frontiers in Psychology, 10*(MAR), 500. https://doi.org/10.3389/fpsyg.2019.00500.

Karacan, I., Rosenbloom, A. L., Londono, J. H., Williams, R. L., & Salis, P. J. (1975). Growth hormone levels during morning and afternoon naps. *Behavioral Neuropsychiatry, 6*(1–12), 67–70.

Knoblauch, V., Kräuchi, K., Renz, C., Wirz-Justice, A., & Cajochen, C. (2002). Homeostatic control of slow-wave and spindle frequency activity during human sleep: Effect of differential sleep pressure and brain topography. *Cerebral Cortex, 12*(10), 1092–1100. https://doi.org/10.1093/cercor/12.10.1092.

Konrad, C., Dirks, N. D., Warmuth, A., Herbert, J. S., Schneider, S., & Seehagen, S. (2018). Sleep-dependent selective imitation in infants. February 1 *Journal of Sleep Research*, e12777. https://doi.org/10.1111/jsr.12777.

Kurdziel, L., Duclos, K., & Spencer, R. M. C. (2013). Sleep spindles in midday naps enhance learning in preschool children. *Proceedings of the National Academy of Sciences, 110*(43), 17267–17272. https://doi.org/10.1073/pnas.1306418110.

Kurth, S., Dean, D. C., Achermann, P., O'Muircheartaigh, J., Huber, R., Deoni, S. C. L., … LeBourgeois, M. K. (2016). Increased Sleep Depth in Developing Neural Networks: New Insights from Sleep Restriction in Children. *Frontiers in Human Neuroscience, 10*, 1–9. https://doi.org/10.3389/fnhum.2016.00456.

Kurth, S., Lassonde, J. M., Pierpoint, L. A., Rusterholz, T., Jenni, O. G., McClain, I. J., … LeBourgeois, M. K. (2016). Development of nap neurophysiology: preliminary insights into sleep regulation in early childhood. *Journal of Sleep Research, 25*(6), 646–654. https://doi.org/10.1111/jsr.12427.

Lokhandwala, S., & Spencer, R. M. C. (2020). Slow wave sleep in naps supports episodic memories in early childhood. *Developmental Science, 24*(2). https://doi.org/10.1111/desc.13035.

Lopez-Calderon, J., & Luck, S. J. (2014). ERPLAB: An open-source toolbox for the analysis of event-related potentials. *Frontiers in Human Neuroscience, 8*(1 APR), 213. https://doi.org/10.3389/fnhum.2014.00213.

Mantua, J., & Spencer, R. M. C. (2017). Exploring the nap paradox: are mid-day sleep bouts a friend or foe? *Sleep Medicine, 37*, 88–97. https://doi.org/10.1016/j.sleep.2017.01.019.

Mason, G. M., Kirkpatrick, F., Schwade, J. A., & Goldstein, M. H. (2019). The role of dyadic coordination in organizing visual attention in 5-month-old infants. *Infancy, 24*(2), 162–186. https://doi.org/10.1111/infa.12255.

Mason, G. M., Lokhandwala, S., Riggins, T., & Spencer, R. M. C. (2021). Sleep and human cognitive development. *Sleep Medicine Reviews, 57*, Article 101472. https://doi.org/10.1016/j.smrv.2021.101472.

Mcclain, I. J., Lustenberger, C., Achermann, P., Lassonde, J. M., Kurth, S., & Lebourgeois, M. K. (2016). Developmental Changes in Sleep Spindle Characteristics and Sigma Power across Early Childhood. *Neural Plasticity, 2016*, 1–9. https://doi.org/10.1155/2016/3670951.

Mölle, M., Bergmann, T. O., Marshall, L., & Born, J. (2011). Fast and slow spindles during the sleep slow oscillation: Disparate coalescence and engagement in memory processing. *Sleep, 34*(10), 1411–1421. https://doi.org/10.5665/SLEEP.1290.

Ohayon, M. M., Carskadon, M. A., Guilleminault, C., & Vitiello, M. V. (2004). Meta-Analysis of Quantitative Sleep Parameters From Childhood to Old Age in Healthy Individuals: Developing Normative Sleep Values Across the Human Lifespan. *Sleep, 27*(7), 1255–1273.

Richmond, J., & Nelson, C. A. (2007). Accounting for change in declarative memory: A cognitive neuroscience perspective. *Developmental Review, 27*(3), 349–373. https://doi.org/10.1016/j.dr.2007.04.002.

Sadeh, A. (2004). A brief screening questionnaire for infant sleep problems: validation and findings for an Internet sample. *Pediatrics, 113*(6), e570–e577. https://doi.org/10.1542/peds.113.6.e570.

Sadeh, A., Acebo, C., Seifer, R., Aytur, S., & Carskadon, M. A. (1995). Activity-based assessment of sleep-wake patterns during the 1st year of life. *Infant Behavior and Development, 18*(3), 329–337. https://doi.org/10.1016/0163-6383(95)90021-7.

Seehagen, S., Konrad, C., Herbert, J. S., Schneider, S., & Heller, H. C. (2015). Timely sleep facilitates declarative memory consolidation in infants. *Proceedings of the National Academy of Sciences, 3*(5), 1625–1629. https://doi.org/10.1073/pnas.1414000112.

Seehagen, S., Zmyj, N., & Herbert, J. S. (2019). Remembering in the Context of Internal States: The Role of Sleep for Infant Memory. *Child Development Perspectives*, 1–6. https://doi.org/10.1111/cdep.12321. epub ahead of print.

Simon, K. N. S., Werchan, D., Goldstein, M. R., Sweeney, L., Bootzin, R. R., Nadel, L., … Gómez, R. L. (2017). Sleep confers a benefit for retention of statistical language learning in 6.5 month old infants. *Brain and Language, 167*, 3–12. https://doi.org/10.1016/j.bandl.2016.05.002.

Siren-Tiusanen, H., & Robinson, H. A. (2001). Nap schedules and sleep practices in infant-toddler groups. *Early Childhood Research Quarterly, 16*(4), 453–474. https://doi.org/10.1016/S0885-2006(01)00119-3.

Sloetjes, H., & Wittenburg, P. (2008). Annotation by category-ELAN and ISO DCR. *Proceedings of the 6th International Conference on Language Resources and Evaluation*, 816–820.

Slone, L. K., & Johnson, S. P. (2015). Infants' statistical learning: 2- and 5-month-olds' segmentation of continuous visual sequences. *Journal of Experimental Child Psychology, 133*, 47–56. https://doi.org/10.1016/j.jecp.2015.01.007.

Solano, A., Riquelme, L. A., Perez-Chada, D., & Della-Maggiore, V. (2021). Local coupling between sleep spindles and slow oscillations supports the stabilization of motor memories. *BioRxiv*. https://doi.org/10.1101/2020.08.24.264697, 2020.08.24.264697.

Spencer, R. M. C. (2021). The role of naps in memory and executive functioning in early childhood. *Advances in Child Development and Behavior, 60*, 139–158. https://doi.org/10.1016/BS.ACDB.2020.08.004.

Squire, L. R. (2004). Memory systems of the brain: A brief history and current perspective. *Neurobiology of Learning and Memory, 82*(3), 171–177. https://doi.org/10.1016/j.nlm.2004.06.005.

Squire, L. R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science, 253*, 1380–1386. https://doi.org/10.1126/science.1896849.

Staresina, B. P., Bergmann, T. O., Bonnefond, M., Van Der Meij, R., Jensen, O., Deuker, L., … Fell, J. (2015). Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nature Neuroscience, 18*(11), 1679–1686. https://doi.org/10.1038/nn.4119.

Stickgold, R. (2009). How do I remember? Let me count the ways. October *Sleep Medicine Reviews, 13*(5), 305–308. https://doi.org/10.1016/j.smrv.2009.05.004.

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Medicine Reviews, 10*(1), 49–62. https://doi.org/10.1016/j.smrv.2005.05.002.
Tribble, R. C., Dmitrieva, J., Watamura, S. E., & LeBourgeois, M. K. (2015). The cortisol awakening response (CAR) in toddlers: Nap-dependent effects on the diurnal secretory pattern. *Psychoneuroendocrinology, 60*, 46–56. https://doi.org/10.1016/j.psyneuen.2015.05.009.
Weissbluth, M. (1995). Naps in Children: 6 Months-7 Years. *Sleep, 18*(2), 82–87.