Memory in 3 month old infants benefits from a short nap

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Abstract: A broad range of studies demonstrate that sleep has a facilitating role in memory consolidation (see Rasch & Born, 2013). Whether sleep dependent memory consolidation is also apparent in infants in their first few months of life has not been investigated. We demonstrate that 3 month old infants only remember a cartoon face approximately 1.5 - 2 hours after its first presentation when a period of sleep followed learning. Furthermore, habituation time, i.e. the time to become bored with a stimulus shown repetitively, correlated negatively with the density of infant sleep spindles, implying that processing speed is linked to specific electroencephalographic components of sleep. Our findings show that without a short period of sleep infants have problems remembering a newly seen face, that sleep enhances memory consolidation from a very early age, highlighting the importance of napping in infancy, and that infant sleep spindles may be associated with some aspects of cognitive ability.

Keywords: sleep, nap, habituation, infants, memory consolidation

Research highlights

- This study provides the first demonstration of sleep related memory consolidation in 3-month-olds.
- A short nap is beneficial in 3-month-old infants for remembering cartoon faces after a 2 hr delay.
- Sleep spindles in 3-month-olds are associated with rate of habituation to new stimuli, indicating a possible association with processing speed.

Introduction

Infants spend most of their days sleeping yet acquire an enormous amount of information, suggesting that their rapid development and their sleep may not be independent of each other. Recent studies show that if infants and pre-schoolers have a nap after learning, they are better at remembering locations of visual stimuli (Kurdziel, Duclos, & Spencer, 2013), actions performed on puppets (Seehagen, Konrad, Herbert, & Schneider, 2015), object-label associations (Friedrich, Wilhelm, Born, & Friederici, 2015; Horvath, Myers, Foster, & Plunkett, 2015), along with being better at generalising knowledge to similar but novel stimuli (Friedrich et al., 2015; Gomez, Bootzin, & Nadel, 2006; Horvath, Liu, & Plunkett, 2016; Hupbach, Gomez, Bootzin, & Nadel, 2009) and at the retention of statistical word segmentation (Simon et al., 2016). In some studies (Hupbach et al., 2009; Kurdziel et al., 2013; Seehagen et al., 2015), for children tested on the following day, only those who had napped within a short period after the learning event remembered the original stimuli; indicating that the nap group's improved recall was long-lasting and that night time sleep had not restored the performance of the wake group. Furthermore, more frequent daytime naps are positively associated with later vocabulary development (Horvath & Plunkett, 2016). These data suggest that the frequent naps occurring in infancy are necessary to efficiently consolidate memories. The youngest age group in which sleep-dependent memory consolidation has been demonstrated is six months (Seehagen et al., 2015). By this age, not just sleep duration decreases remarkably but sleep structure (including the proportion and the frequency composition of different sleep stages) goes through substantial changes and becomes more adult-like. Three month of age is an interesting midpoint to study as the sleep stages known from adult literature just becomes visually distinctive in addition to sleep spindles which becomes apparent around this age (Grigg-Damberger et al. 2007). However, the properties of sleep spindles (Scholle, Zwacka & Scholle, 2007) and the spectral composition of sleep is different from adults and older infants

and children (Jenni, Borbely & Achermann, 2004; Sankupellay, Wilson, Heussler, Parsley, Yuill & Dakin, 2011). It remains unclear therefore whether the high rate of napping in much younger infants whose memory consolidation is more fragile (Mullally & Maguire, 2014) and sleep structure is different also facilitates sleep-memory consolidation and whether similar mechanisms are involved.

Sleep spindles, waxing and waning 10-15 Hz oscillations appearing in non-rapid eye movement sleep, have been proposed to play a role in neuronal modifications (Rosanova & Ulrich, 2005; Steriade, 1999) and in the transfer of information between different brain regions (Buzsaki, 1996). They have been related to learning efficiency (Schabus et al., 2006), general cognitive ability/intelligence (Bodizs et al., 2005; Bodizs, Gombos, Ujma & Kovacs, 2014; Lustenberger, Maric, Durr, Achermann, & Huber, 2012; Ujma et al. , 2014; Schabus et al., 2006) and sleep-dependent improvement in memory performance (Clemens, Fabo, & Halasz, 2005, 2006; Gais, Molle, Helms, & Born, 2002; Schabus et al., 2004; Tamminen, Payne, Stickgold, Wamsley, & Gaskell, 2010) in adults. However, their importance is still not clear especially for children, as some measures of sleep spindles (e.g. peak frequency) show a negative association, while others (e.g. sigma power, number of fast spindles, spindle activity of slow spindles) correlate positively with intelligence (Chatburn et al., 2013; Geiger et al., 2011; Hoedlmoser et al., 2014; Ujma, Sandor, Szakadat, Gombos & Bodizs, 2016). Only two studies have investigated the relationship between learning and sleep spindles in younger children, one in 9-12 month infants (Friedrich et al., 2015) and one in 3-5 year old pre-schoolers (Kurdziel et al., 2013). Sleep spindles (density and sigma power) correlated positively with the sleep-dependent improvement in performance. Whether infants younger than 9 months show a similar relationship between learning and sleep spindling and whether this relationship remains stable throughout early development has yet to be explored.

Our main goal was to investigate whether the memory of 3 month old infants benefits from a daytime nap. In order to investigate this question, we compared memory performance of two groups: one who napped after learning and another who stayed awake. Infants' recall performance was assessed with a method often used with preverbal infants, the habituation-dishabituation paradigm with a visual paired comparison (VPC) task (e.g. Burbacher & Grant, 2012). Following several repetitions of a stimulus, infants become habituated, lose interest, and consequently, prefer to look at a novel stimulus. With a time lag between habituation and the visual comparison (which can contain either sleep or wake), we can test whether infants remember the old stimulus and whether there are any differences between the wake and the nap groups. If infants remember, they will show novelty preference and look significantly longer to the novel stimulus compared to the old one.

In our experiment, cartoon figures depicted in Fig. 1. were used as stimuli. As there are individual differences in habituation rates, we implemented a new automatic habituation procedure involving automated eye-tracking to ensure that every infant habituated to the same level. We defined habituation on the basis of previously set criteria (Pascalis, de Haan, Nelson, & de Schonen, 1998). Automatic eye tracking with gaze contingent presentation allowed us to avoid the possibility of experimental error and bias.

Our second goal was to investigate whether learning in very young infants is related to sleep spindling, as has been reported for adults. In the adult literature, there is a debate as to whether correlations between cognitive performance and sleep spindles reflect trait-like associations (i.e. general cognitive ability) or learning per se. To investigate these issues further, we calculated Spearman correlations between sleep spindle density (number of sleep spindles per minute) and habituation time as well as novelty preference. Novelty preference is a measure of recognition memory which is a good predictor of later IQ (Slater, 1995). If there is an association with novelty preference, it could be because sleep spindles correlate with IQ (a

trait-like relationship) or with actual learning in the specific test (a state-like relationship). However, an association with habituation time, which is a measure of information processing speed) would suggest a trait-like association. Due to our use of individualised habituation criteria, we may reasonably assume that all infants learnt to recognise the new stimulus. Therefore, habituation time cannot indicate a difference in recognition memory[1]. Moreover, it is unlikely that faster processing speed would cause an immediate change in sleep spindle density.

We hypothesized that 3 month old infants will only be able to remember the cartoon face if they had a nap after habituation. Furthermore, we expected to find a correlation between sleep spindle density and both novelty preference and habituation time.

[1] Note that a test of recognition immediately following habituation would potentially contaminate a further test of recognition after a prolonged period of wake or sleep. Therefore, we avoided such potential contamination and only tested for recognition after a delay.

Fig. 1. Experimental stimuli used in the VPC trials.

Materials

Participants

Sixty-seven, 3 month old infants took part in the current experiment. All infants were recruited via a database of parents who previously expressed an interest in taking part in developmental research. Of the original 67 infants, 22 were excluded due to being more than 3 weeks preterm (n = 3), chronic health problems (n = 1), failed calibration with the eye tracking equipment (n = 11), fussiness (n = 3), inattention (n = 3) or experimental error (n = 1). As such, the final sample contained 45 infants (30 female) born to term (> 37 weeks), ranging from 86 – 122 days old (M = 100.2, SD = 8.56; corrected age[2] M = 97.67, SD = 13.1). No infants had apparent problems with vision or known history of developmental disorders or delays. Originally, sample size was determined as approximately 30 infants on the basis of our previous studies in older age groups. Due to the difficulties with obtaining good quality sleep EEG data, we continued recruiting additional 15 infants for our sleep group to be able to perform correlational analyses. Infants received a small gift for participation. Excluded infants did not differ from included ones in parental education (U (34) = 84, p = .256), maternal depression (U (42) = 162, p = .822) score, nor habituation time (t (51) = 1.292, p = .202).

Stimuli

Presentation of habituation and novel stimuli were always preceded by an attention grabbing rotating green star with a red outline. The star was edited in Adobe Photoshop (CS5, Adobe Systems Inc.) and presented centrally upon a white background, with a presentation size of 13cm x 13cm (600 x 600 pixels).

One of two possible cartoon characters (red haired boy or blue haired girl) with emphasised facial features were used as habituation stimuli, with the unused character serving

[2] Infants age in days minus the number of days the infant was preterm

as the novel stimulus during VPC test trials. Both characters were created using an online programme (www.doppelme.com) and edited in Adobe Illustrator (CS5, Adobe Systems Inc.) to create final images measuring 14.7cm x 25.5cm (600 x 1047 pixels), presented upon a white background. During both habituation and VPC trials, all characters were displayed 25cm apart on the left and right side of the display screen.

Eye tracking

Presentation of the above stimuli took place using custom built routines with PresentMate software in MATLAB (version 7.14, R2012a, The MathWorks, Inc, Natick, MA), and displayed on a 23-inch (51cm x 29cm) widescreen TFT monitor. Eye movements during both habituation and VPC trials were concurrently recorded using a Tobii TX300 Eye Tracker at a 60Hz sampling rate for 10 infants because of calibration failure at a 300Hz sampling rate. The remaining infants were originally recorded using a 300Hz sampling rate and data were later downsampled to 60Hz.

Questionnaires

Parental education: a question required both parents to divulge information relating to their highest educational attainment on a seven-point scale, ranging from GCSE's to a professional doctorate (e.g. MD).

In order to assess maternal depressive symptomology the 20-item Epidemiological Studies Depression Scale (CES-D) (Radloff, 1977) was utilised.

Finally, information pertaining to infant sleep behaviour was obtained using the Sleep and Naps Oxford Research Inventory (SNORI). The SNORI (Horvath et al., 2016) includes a 10-day sleep diary requiring parents to indicate within 15-minute accuracy whether their child was awake or asleep over a discrete 24-hour period.

Procedure

The research was approved by The University of Oxford Research Ethics Committee (MSD/IDREC/C2/2012/11). The study utilised a between-participants design comprised of two levels, with infants assigned to either a 'wake' or 'nap' group. Allocation to such groups was based upon a pre-study recruitment procedure where parents were informed that their child was invited into the experiment as pair of *either* the wake *or* nap group. Wake group infants were invited into the laboratory after their usual nap to avoid sleeping, whereas nap group infants came in before their usual nap time to increase the chance for falling asleep. Even though this approach resulted in a difference between times of testing between groups ($t(43) = -3.527$, $p = .001$, M_w = 13:42, SD_w = 1:07, M_s = 15:21, SD_s = 1:42), it enabled avoidance of sleep deprivation of infants which we deemed of greater importance for the current study.

Prior to the agreed testing date, all parents were given a detailed study description and asked to complete questionnaires. Upon study commencement, parents were informed of their right to withdraw and asked to sign a consent form. All infants subsequently took part in the habituation procedure.

Habituation trials took place in a grey recording booth, with a display monitor, speakers and eye tracking equipment mounted upon the wall. Infants sat on their parent's lap facing the centre of the display monitor at a viewing distance of approximately 65cm. Prior to the commencement of habituation trials, all infants eye movements were calibrated with the above mentioned eye tracking equipment. This involved the presentation of a rotating yellow and red star appearing at nine different positions on the monitor. Calibration was considered successful when two good calibration points on each side of the screen were obtained. Once successfully calibrated, the infant's attention was drawn to the centre of the screen using the rotating

attention grabbing star. This was displayed until infant's fixation fell within the star's target area (13cm x 13cm area). If no fixation was recorded after two seconds, the green star could be replayed or trials aborted. Once fixation was established, two identical cartoon characters (either the red haired boy or blue haired girl) appeared on either side of the display monitor. The cartoon characters remained on screen until the infant's fixation fell outside of the two character's target area (14.7 cm x 25.5cm) for more than two continuous seconds, or until the maximum looking time of an individual trial (20 seconds) was reached. Following a one second inter-trial interval, the attention grabbing green star reappeared followed by the presentation of the two identical cartoon characters previously seen. Trials continued in this manner until either the maximum eight trials was reached, or looking time at the habituation stimuli, from the fourth trial onward, was less than half of the mean looking time during the first three trials (adapted from (Pascalis et al., 1998)). For example, a mean looking time at the habituation stimuli of 14 seconds during the first three trials would create a critical threshold of seven seconds. Looking times of less than this critical threshold, from the fourth trial onwards, would lead to cessation of habituation trials and count as a successful habituation. The habituation procedure is illustrated in Fig. 2.

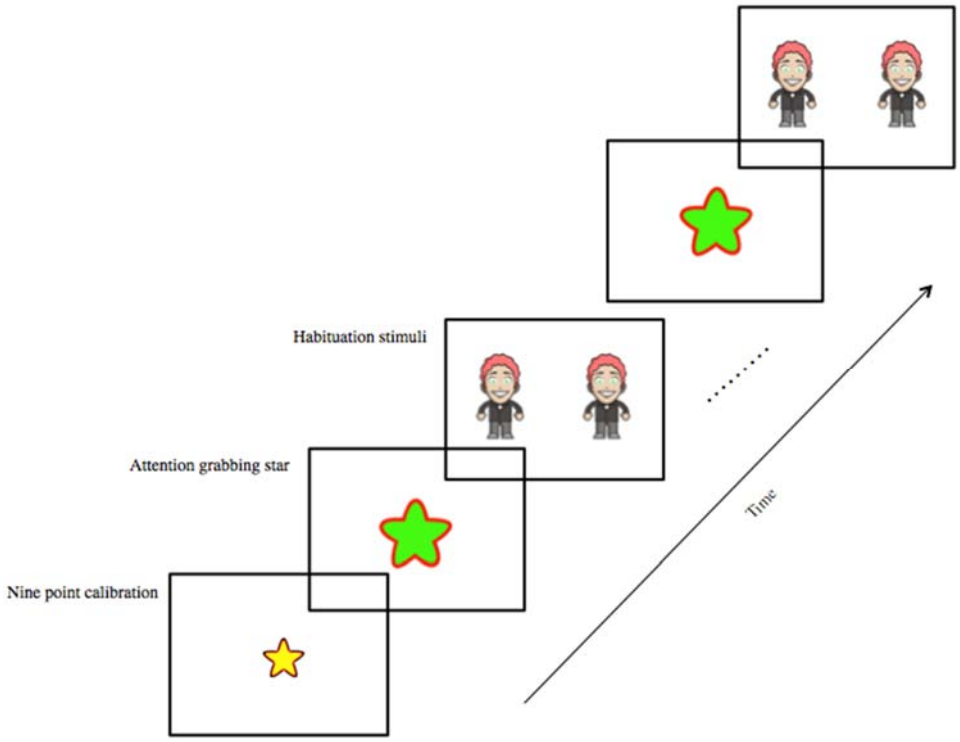

Fig. 2. Habituation trial procedure.

Following successful habituation, infants were allocated to their pre-assigned wake or nap group condition. Those allocated to the wake group engaged in wake promoting activities (e.g. play) in order to discourage spontaneous napping. Sleep group infants underwent polysomnographic electrode application which took about 10 minutes. They were then transported to a climate controlled sleep room containing the polysomnographic recording hardware and a cot.

After an interval of approximately 1.5 hours (M = 98.93 min, SD = 21.97 min), all infants took part in the VPC testing procedure. Sleep group infants were given an approximate 15 minute interval between waking and test trials, to protect against sleep inertia. Testing took place in the same room as habituation, and began with a calibration procedure identical to that previously mentioned. Following establishment of fixation within the attention grabber's target area, VPC test trials began. During test trials, cartoon characters appeared simultaneously for a fixed twenty-second time limit. One of the cartoons presented was the previously habituated cartoon character. In contrast to habituation trials however, the second cartoon character was not identical, but instead the previously unseen novel character. After a two second inter-trial interval, presentation of the attention grabbing green star and cartoon characters was repeated for a further 20 seconds, counterbalancing for side of novel stimuli presentation. The order of the trials was random within infants. The testing procedure can be seen in Fig. 3.

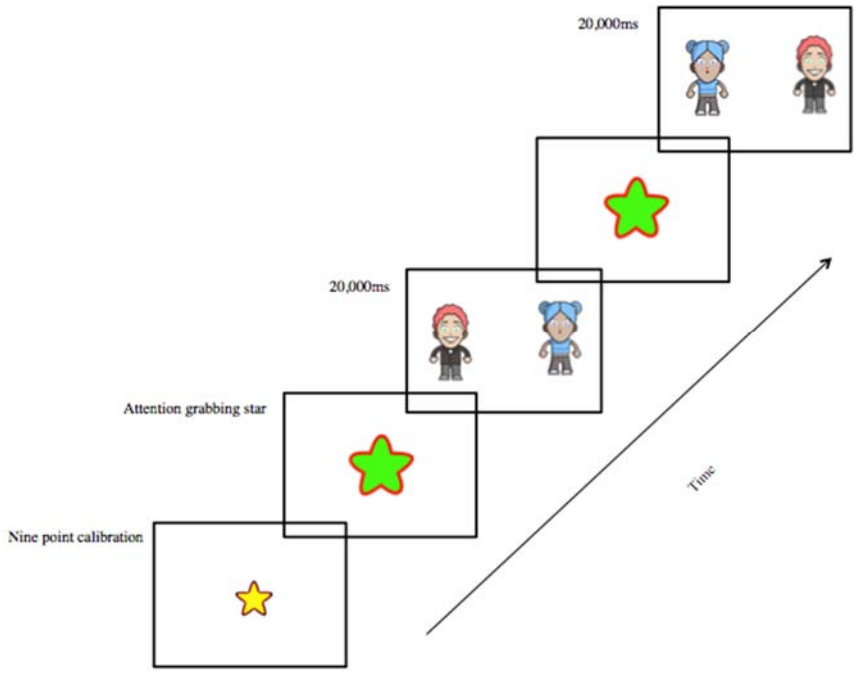

Fig. 3. Visual paired comparison test trials procedure.

Data processing of eye tracking data

Custom built MATLAB routines were used to calculate the number of trials needed for each infant to reach habituation criterion; the length of looking on each individual habituation trial, and the critical threshold of looking time at which habituation trials would cease. In addition, looking behaviour during VPC test trials was analysed using custom built MATLAB routines. Raw gaze data was smoothed using a 3-point median filter, with fixations identifiable based within a 35-pixel radius over a period of 66.7ms of the smoothed gaze data. Fixations towards the novel or habituated character were calculated as such if they fell within the 25.5cm x 14.7cm (600 x 1047 pixels) target area of each stimulus. The proportion of time spent fixating the novel item was then divided by the total time fixating both the novel and familiar characters to create a 'novelty preference score'. Two of the infants in the nap group failed to provide valid data on the test trials due to calibration problems.

Polysomnography

Polysomnographic recordings were conducted using silver chloride electrodes at sites F3, F4, C3, C4, O1 and O2 referenced to Cz, based upon the international 10-20 system. All electrodes were fixed to the scalp using Nihon Kohden Elefix Z-401CE paste for EEG and secured with a sterile absorbent gauze bandage over each electrode site. The recording signal was amplified and digitalised with a NuAmps 7181 digital EEG amplifier with 1024 Hz/channel sampling rate and filtered with 0.5 - 40 Hz bandpass filter.

Processing of EEG data and sleep spindle detection

Of the 28 infants in the nap group, the EEG data of 15 were included in our analyses. The data for 13 infants were excluded because of poor data quality (10), experimenter error (1) or equipment problems (2). Sleep stages were scored on the basis of the EEG according to

standard criteria and artefacts were manually removed using 4 s epochs. Sleep spindles were detected with the Individual Adjustment Method (Bodizs, Kormendi, Rigo, & Lazar, 2009; Ujma et al., 2015), which uses individual frequency bands and amplitude criteria for sleep spindle detection. We performed spectral analysis for all channels (fast Fourier transformation, Hanning window, 20 s epochs) with 0.25 Hz frequency bins between 1 - 48 Hz. On the basis of Kurth et al. (Kurth et al., 2010), we restricted our analysis to absolute spectra of specific frequency bands: slow waves (1-4.5 Hz), theta (4.75 – 7.75 Hz), alpha (8 – 9.75 Hz), sigma (10 – 15 Hz) and beta (20 – 25 Hz).

Results

To examine whether the nap and wake groups behaved differently, we compared the novelty preference after habituation to chance (50%) with one-sample t-test in the two groups separately. Our initial analysis showed that averaging the two trials used during testing nullifies novelty preference. The most likely reason – especially considering the long duration of the testing trials (20s) – is that infants become habituated to the novel stimulus during the first trial[3]. Therefore, analysis of looking behaviour during the first VPC test trial only is highlighted. Both groups' novelty preferences were assessed for a significant deviation from chance looking behaviour using the same one-sample t-tests previously described. The results indicated that novelty preference in wake group infants did not significantly differ from the hypothetical 50% value (t (16) = 0.716, p = .484, M = 56.47, 95% CI [-12.68, 25.62]). However, infants who napped between habituation and the VPC test session demonstrated novelty preferences that significantly differed from chance (t (24) = 2.847, p = .009, *Cohen's d* = 1.16,

[3] Further justifying our decision to avoid a recognition test immediately following habituation.

M = 67.13, *95% CI* [4.74, 29.52]), indicating that during the first VPC trials, these infants preferred to look towards novel stimuli.

To ensure there were no differences between each group upon study enrolment, wake and nap group infants were compared on several variables. The two groups did not differ systematically in terms of age (t(43) = -1.356, p = .182) nor corrected age (t(43) = -1.458, p = .152). Responses to the CES-D and parental education were analysed in a subset (wake: 16, sleep: 15 and wake: 16, sleep: 9, respectively) of our sample who provided valid data to ensure there were no significant between group differences (Table 1). Preliminary assumption testing indicated a significant deviation from normality in responses for both variables. As such, the non-parametric Mann-Whitney U test was utilised. Results indicated no significant differences between the wake and nap group infant's maternal depressive symptomology (*U* (31) = 108.00, *p* = .654) nor parental education (*U* (25) = 71.00, *p* = .978). We also compared a subset of the two groups (those were included who fully completed the SNORI) along sleep variables obtained from the SNORI found no significant difference in night time (t(18) = 1.363, p = .19), daytime sleeping (r(18) = -.903, p =.378), nor awakening time (t(18) = -.687, p = .501) during night.

Table 1.

Demographic and sleep variables by group.

	Age (days)	Corrected age (days)	CES-D	Parental education	Night time sleep (min)	Daytime sleep (min)	Awakening time during night (min)
	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)
Wake	98 (7.8)	94.06 (9.61)	7.88 (6.93)	8.44 (2.25)	604.65 (46.95)	191.71 (46.95)	125.49 (52.14)
Sleep	101.54 (8.86)	99.86 (14.55)	6.4 (5.94)	8.89 (1.36)	535.33 (145.92)	236.59 (139.94)	143.52 (63.05)

Note: Means (M) and standard deviations (SD) for responses to age, corrected age, CES-D, parental education and sleep variables separated by group.

The amount of time between habituation and the VPC test session, the number of trials it took to reach habituation, and cumulative looking time at the cartoon character during habituation trials are summarised in Table 2. To be sure that looking behaviour was not influenced by differential experiences with the above variables, possible between group differences were assessed. Results indicated no significant differences in terms of the time between habituation and VPC test sessions ($t(43) = -1.577$, $p = .122$), the number of trials to reach habituation criterion ($t(43) = -.552$, $p = .584$), or cumulative looking time at the habituation stimuli ($t(42.363) = -1.894$, $p = .065$), suggesting that wake and nap groups experience of habituation was not significantly different. The numerical difference is most probably due to scheduling differences and the different alertness of the groups. However, with individualizing habituation criteria, it is not likely that the two groups became habituated to different levels. There was no significant correlation between habituation time and novelty preference ($r (43) = .074$, $p = .636$), making it unlikely that the novelty preference in the sleep group was the consequence of the numerically higher habituation time.

Table 2.

Comparing habituation between wake and nap groups.

Group	Time between habituation and VPC test session (*minutes*) *M* (*SD*)	Trials to habituation *M* (*SD*)	Cumulative habituation time (*seconds*) *M* (*SD*)
Wake	91.47 (9.36)	5.06 (1.39)	33.58 (29.54)
Nap	101.93 (26.24)	5.32 (1.63)	54.25 (43.53)

Note: Means (M) and standard deviations (SD) for intersession interval trials to habituation and cumulative habituation time by group.

To ensure that sex or habituation stimuli did not influence looking behaviour a 2 (sex) x 2 (habituation stimuli) between-participants ANOVA using first trial novelty preference was conducted in the two groups. Results indicated no significant main effect of either sex (F (1, 43) = 0.019, p = .892), or habituation stimuli (F (1, 43) = 0.703, p = .407), nor a significant interaction between sex and habituation stimuli (F (1, 43) = 0.28, p = .599), suggesting that these variables did not influence subsequent looking behaviour. These data clearly demonstrate that only the nap group showed novelty preference, indicating that a nap was necessary for successful consolidation of the habituated cartoon figure.

Given that novelty preference was only evident in nap group infants during the first VPC trial alone, we continued to use the data of the first novelty preference trial to investigate the relationship between spindles and learning. Slow and fast spindles were analysed separately based on their different ontogenetic pattern (Hoedlmoser et al., 2014). We performed correlational analyses calculating Spearman correlational coefficients to avoid data points with larger values having a disproportionally large effect due to our small sample size. There was no significant correlation between novelty preference and either slow or fast spindle density at any of the electrode sites. However, habituation time correlated negatively with slow spindle density on F3 (rho (15) = -.65, p = .009), F4 (rho (15) = -.529, p = .043) and C4 (rho (15) = -.55, p = .034) electrodes, indicating that those who had higher sleep spindle density processed the visual information faster. Importantly, after controlling for multiple testing (Benjamini & Hochberg, 1995), only the correlation for F3 electrode remains significant. Correlations are shown in Fig. 4. These correlations point to the possibility that general cognitive ability at 3 months, as measured by habituation time, is related to sleep spindle density. Descriptive

statistics of sleep and sleep spindle parameters as well as correlations are provided in Tables 3-5. There were no significant correlations between the length of sleep, different sleep stages or the different SNORI variables and habituation time or novelty preference.

In an exploratory analysis, we examined whether different frequency bands of the EEG spectra correlated significantly with habituation time or novelty preference. There were no significant correlations in any of the frequency bands.

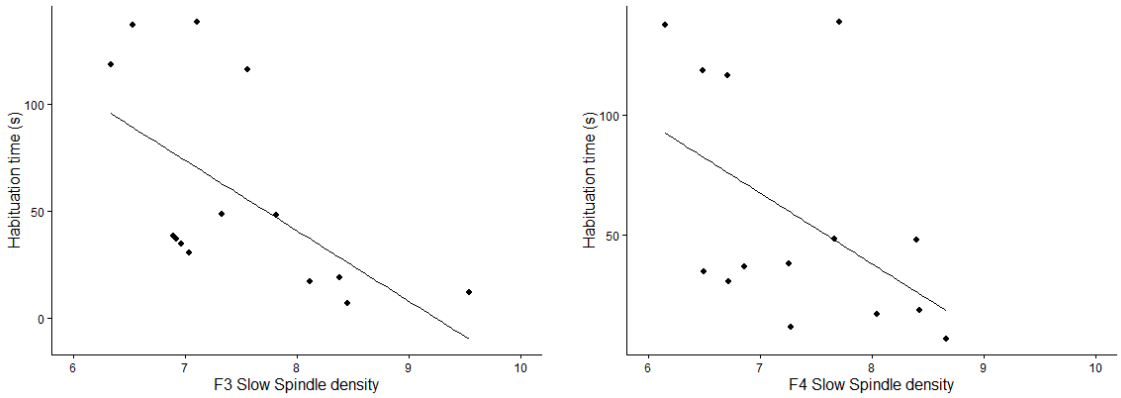

Fig. 4. Associations between sleep spindles and habituation time. We found significant negative correlations between habituation time and slow spindle density on F3 and F4 electrodes although only the correlation on F3 remained significant after controlling for multiple comparisons. Raw data are presented.

Table 3.

Correlations of habituation time and novelty preference with sleep spindle density

		F3		F4		C3		C4		O1		O2	
		Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast
Novelty preference	rho	-.007	.168	.318	-.261	-.246	-.032	-.114	-.014	-.114	-.289	-.79	-.118
	p	.98	.55	.243	.348	.376	.909	.685	.96	.685	.296	.781	.676
Habituation time	rho	-.65	.161	-.529	.029	-.364	.296	-.55	.114	-.436	.096	-.468	.239
	p	.009	.567	.043	.919	.182	.283	.034	.685	.104	.732	.079	.39

Table 4.

Sleep parameters

Sleep Time (minutes) *M* (*SD*)	NREM 2 (%) *M* (*SD*)	SWS (%) *M* (*SD*)	REM* (%) *M* (*SD*)
41.87 (21.54)	54.44 (22.43)	34.99 (25.83)	4.44 (9.67)

Note: Means (M) and standard deviations (SD) for sleep time and proportion of time within each sleep stage.

NREM – non-rapid eye movement sleep, SWS – slow wave sleep, REM – rapid eye movement sleep.

*Only four infants have reached REM sleep.

Table 5.

Sleep spindle density

	F3		F4		C3		C4		O1		O2	
	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast
M	7.39	6.07	7.25	5.96	7.65	5.98	7.52	6.07	7.82	6.12	7.88	6.06
SD	0.94	0.98	0.86	0.94	0.95	1.06	1.05	0.88	1.06	0.93	0.9	0.98

Note: Means and standard deviations of slow and fast sleep spindle density (min-1).

Discussion

To the best of our knowledge, this is the first study showing sleep-dependent memory consolidation in infants as young as 3 months old, and an association between sleep spindles and general cognitive ability in infancy. In accordance with our hypothesis, novelty preference differed significantly from chance only in the nap group, indicating that these infants were still habituated to the old stimulus. The wake group spent similar amounts of time looking at both stimuli, suggesting they were not habituated to the old one anymore. Furthermore, we found significant negative correlations between habituation time and slow sleep spindle density on F3, F4 and C4 electrodes. However, after controlling for multiple comparisons only the correlation for F3 remained significant. Interestingly, we did not find a significant correlation with novelty preference (actual test performance) and sleep spindle density.

Our results indicate that infants need a nap to be able to recognize the familiar cartoon face approximately 1.5 – 2 hours after first exposure. A plausible explanation is that infants' immature memory system makes them dependent on frequent daytime naps because of its limited capacity (Kurdziel et al., 2013; Mullally & Maguire, 2014; Seehagen et al., 2015). We suggest two factors that may play a contributory role. First, infants' short term storage capacity may be small. Thus, to prevent saturation and interference, more frequent sleep periods are needed to empty or refresh these stores. Second, consolidation may be slower or less efficient during wake in contrast to adults and older children who are able to preserve memories for longer periods, either because they can consolidate memories during wake or because they have a larger storage capacity. The fact that we did not find novelty preference in the second trial of testing supports this hypothesis, together with the findings of Simon et al. (2016) in which sleep-dependent enhancement was only apparent in the first block of testing. Simon at al. (2016) claimed that the testing stimuli may have interfered with the learned material, which may have been the case in this study as well. In sum, these findings suggest that consolidation is weak at these early ages.

It is still a remaining question whether the state of sleep has an additional enhancing role in memory consolidation or it just provides a good protection from interfering stimuli. Unfortunately, on the basis of our design we cannot answer this question. Moreover, it would be unfeasible to do an experiment with infants in which a control group with no stimulation is used, as infants would probably cry or fall asleep. However, we have indirect evidence that sleep is more than a passive prevention of interfering stimulation. First, transcranial direct current stimulation during sleep can improve memory in adults (Marshall, Helgadottir, Molle & Born, 2006). Second, during sleep there are specific biological mechanisms both on a cellular and network level which contribute to effective consolidation (for review see Korte & Schmitz, 2016). Although it is not possible to exclude the possibility of interference on the basis of our experiment, considering evidence from other studies we suggest that sleep has an active role in memory consolidation.

Our findings highlight that even at 3 months of age a relationship exists between specific sleep parameters, i.e. sleep spindles, and specific aspects of cognitive ability even though sleep spindles have not yet reached their adult form. As some studies suggest that habituation time may be an indicator of later intelligence (Slater, 1995), our results reflect an association between sleep spindles and some aspects of early general cognitive abilities. Further studies should examine, however, whether sleep spindles can be an indicator of later IQ. Interestingly, contrary to our hypothesis, we did not find an association with actual memory performance. In this regard, our results support theories which consider sleep spindling as the reflection of early general intelligence. Furthermore, the lack of an association between novelty preference and sleep spindles may support theories which claim that hippocampus dependent memory consolidation cannot occur earlier than around 18 month due to the prematurity of the hippocampus (Gomez & Edgin, 2015). The temporal synchronization between sleep spindles, hippocampal ripple activity and slow oscillations is thought to provide the neurophysiological

basis for the replay of memories during sleep (Buzsaki, 1996). Considering the immature connections between different hippocampal regions and the cortex (Lavenex & Banta Lavenex, 2013), a different mechanism for sleep-dependent memory consolidation (e.g. synaptic downscaling (Tononi & Cirelli, 2014)) is more likely in infancy (Gomez & Edgin, 2015).

One of the major limitations of our study is that we cannot rule out learning dependent increases in sleep spindle density, as we did not have a control nap which was not preceded by intensive learning. However, using individualized criteria for habituation, our participants were habituated to the same level, and thus, should have learnt the same amount of information. In this regard, habituation time reflects more processing speed than learning. Second, it is not clear from our results whether a similar period of quiet wakefulness would lead to effective consolidation perhaps due to the lack of interfering memories. Nonetheless, keeping infants in quiet wakefulness without any parental interaction would be not tolerated by this age group, hence such an experiment would be unfeasible. Moreover, as we did not test infants immediately after habituation to avoid contamination by the new stimulus, we do not have direct evidence that both groups processed the information to the same level. Although it was statistically not significant, there was a numerically shorter habituation time in the wake group most probably because they were less tired as they had the laboratory testing session after their usual naps. However, the individualized approach for habituation should at least partly overcome this issue. Finally, we have not derived any information regarding how long the consolidated information would last in our participants and how promptly the nap has to follow the learning period to be effective.

Our data suggest that sleep plays a role in memory consolidation in infancy, consistent with previous studies in adults and older children (Rasch & Born, 2013). Moreover, our findings indicate that sleep has a crucial importance at this age, as those infants who did not nap after learning showed no evidence of remembering the stimulus previously shown, whereas those

infants who napped also remembered. We propose that frequent naps during infancy are necessary for the efficient consolidation of information, supporting similar claims made on the basis of longitudinal evidence (Horvath & Plunkett, 2016). Furthermore, our findings point to an association that exists between specific electroencephalographic oscillations i.e. sleep spindles and information processing speed.

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