Sleep spindles during a nap correlate with post sleep memory performance for highly rewarded word-pairs

Sara Studte (M.Sc.)[1]

Emma Bridger (Dr.) [2]

Axel Mecklinger (Prof. Dr.) [1]

[1]Experimental Neuropsychology Unit, Saarland University, Germany

[2]Department of Psychology, Birmingham City University, UK

E-mail addresses:

s.studte@mx.uni-saarland.de

emma.bridger@bcu.ac.uk

mecklinger@mx.uni-saarland.de

Corresponding author:

Sara Studte

Experimental Neuropsychology Unit

Postbox 151150

D-66041 Saarbrücken

Tel.: 0049-681-302-64365

Fax: 0049-681-302-6516

E-Mail: s.studte@mx.uni-saarland.de

Abstract

The consolidation of new associations is thought to depend in part on physiological processes engaged during non-REM (NREM) sleep, such as slow oscillations and sleep spindles. Moreover, NREM sleep is thought to selectively benefit associations that are adaptive for the future. In line with this, the current study investigated whether different reward cues at encoding are associated with changes in sleep physiology and memory retention. Participants' associative memory was tested after learning a list of arbitrarily paired words both before and after taking a 90-minute nap. During learning, word-pairs were preceded by a cue indicating either a high or a low reward for correct memory performance at test. The motivation manipulation successfully impacted retention such that memory declined to a greater extent from pre- to post sleep for low rewarded than for high rewarded word-pairs. In line with previous studies, positive correlations between spindle density during NREM sleep and general memory performance pre- and post-sleep were found. In addition to this, however, a selective positive relationship between memory performance for highly rewarded word-pairs at posttest and spindle density during NREM sleep was also observed. These results support the view that motivationally salient memories are preferentially consolidated and that sleep spindles may be an important underlying mechanism for selective consolidation.

Keywords: Sleep spindles; Nap; Motivation; Reward; Recognition Memory

1. Introduction

One important aspect of language learning, in particular second language learning, is the formation of associations between words. A process thought to be vital to the successful consolidation of memories is sleep (Rasch & Born, 2013). Benefits of sleep have been reported in procedural as well as declarative memory tasks (Fischer & Born, 2009; Fischer, Hallschmid, Elsner, & Born, 2002; Lau, Tucker, & Fishbein, 2010; Marshall, Molle, Hallschmid, & Born, 2004; Tucker et al., 2006; Walker, Stickgold, Alsop, Gaab, & Schlaug, 2005; Wilhelm et al., 2011). It seems that slow oscillations during slow-wave-sleep (SWS) and associated sleep spindles are particularly important for declarative memory consolidation (Born & Wilhelm, 2012; Cox, Hofman, & Talamini, 2012; Gais, Molle, Helms, & Born, 2002; Marshall et al., 2004; Mednick et al., 2013; Saletin, Goldstein, & Walker, 2011; Schmidt et al., 2006). Consequently, SWS and associated physiological mechanisms are presumed to be important for the successful acquisition of new associations which underpin some forms of language learning (Opitz & Friederici, 2004).

In one recent study demonstrating the benefits of SWS and sleep spindles for hippocampus-dependent memories, we used memory tasks with single words and non-related word-pairs to compare the impact of nap sleep on item memory vs. associative memory (Studte, Bridger, & Mecklinger, 2015). In the item memory task, single words were to be judged as learned or new, whilst in the associative task participants were required to distinguish between learnt, learnt but rearranged and new word-pairs. The former test requires only recognition of simple item memory, whereas the ability to retrieve associations between learnt word-pairs is necessary to perform the associative test. A beneficial effect of 90 minutes of nap sleep was only found for associative memory performance, and this manifested as a smaller decrease in associative memory performance over time. Associative recognition memory performance after sleep was also found to be associated with sleep spindle density at frontal sites during

SWS, and performance before sleep was marginally correlated with sleep spindle density at frontal sites during non-REM (NREM) sleep. No corresponding correlations were observed for item memory, which underlines the strong association between associative memory performance and SWS mechanisms.

Not all learnt information is retained after sleep however and which memories benefit from sleep and which do not remains to be fully specified. There is increasing evidence that sleep works as a filter by predominantly strengthening memories that are adaptive or of relevance to the future (Fischer & Born, 2009; Oudiette, Antony, Creery, & Paller, 2013; Saletin et al., 2011; Stickgold & Walker, 2013; van Dongen, Thielen, Takashima, Barth, & Fernández, 2012; Wilhelm et al., 2011). In one model of selective memory consolidation, Stickgold and Walker (2013) assume that consolidation of information will only occur if items are tagged as important during or after encoding. These tags could be induced by task relevance (Saletin et al., 2011; Wilhelm et al., 2011), emotionality (Payne, Stickgold, Swanberg, & Kensinger, 2008) or expected reward (Fischer & Born, 2009; Oudiette et al., 2013). Selective beneficial effects of sleep have been shown for both motor (Fischer & Born, 2009) and declarative memory tasks, such as word paired-associate tasks (Wilhelm et al., 2011) and object location tasks (Oudiette et al., 2013; van Dongen et al., 2012). In one pertinent demonstration of this, Wilhelm and colleagues (2011) asked participants to learn lists of semantically-related word-pair associates before 9 hour retention intervals filled with either sleep or wakefulness. Critically, participants were randomly allocated to be either informed or uninformed that they would be later tested on their memory for these items after the retention interval. Participants who were informed that they would be later tested performed better on the final memory test than their uninformed counterparts, but only if they slept in the retention interval. These participants also demonstrated a robust increase in slow oscillation activity and sleep spindles

during slow-wave-sleep (SWS); again in line with the association between these physiological mechanisms and preserved associative memory.

The preceding considerations of the existent literature strongly indicate that sleep should preserve memory for word-pair associations that are tagged as relevant for the future. Moreover, data repeatedly demonstrating the engagement of SWS mechanisms predicts that the mnemonic benefits for information that undergoes a specific learning experience should be evident even after a 90-minute nap, so long as this is sufficient for individuals to engage in a prolonged phase of SWS. In the current study, all participants learnt a list of word-pairs and were tested on their memory both before and after taking a nap. Critically, half of the word-pairs were preceded by a cue which indicated that later correct performance would be rewarded at a high level; whereas for the remainder, the cue indicated that the reward was relatively low (see Oudiette et al., 2013 for a similar approach to induce motivational salience). The logic behind this manipulation was that these reward cues should make high reward items motivationally more relevant and tagged for selective consolidation during sleep compared to low reward items. This should lead to better memory performance for high- than low-reward items after sleep, manifest as a significantly smaller decline in memory performance for high-rewarded associations over time (Studte et al., 2015). In line with the notion that the physiological variables during NREM/SWS sleep are associated with selective consolidation, however, specific predictions about the relationship between spindle density (SpD) and memory performance were explicitly considered. If a correlation between SpD and memory performance for high but not low rewarded items can be observed, this would provide evidence for a selective role of sleep in memory consolidation, in particular a role for sleep spindles in the selective tagging of memories from a specific learning experience, in our case memories for events with a high motivational value (Murty & Adcock, 2014).

In the current experiment therefore, behavioral and polysomnographic data were used to investigate how reward cues during encoding might interact with the benefits of nap sleep on associative recognition and how this would relate to physiological variables during sleep. A final aspect of the current design was the employment of an associative recognition memory test as was the case in our former study (Studte et al., 2015), in which word-pairs were to be classified as either old, recombined or new. Responses to these categories were used to create two discrimination measures. An old/new discrimination Pr index (PrI- score), calculated by subtracting false alarms to new pairs from the hit rate for old pairs (Snodgrass & Corwin, 1988) was taken to represent item memory performance whilst an associative PrA-score, calculated by subtracting the proportion of recombined pairs incorrectly classified as old (false alarms to recombined) from the hit rate for old pairs, was employed as a measure of recollection/associative memory (Bader, Mecklinger, Hoppstadter, & Meyer, 2010; Kriukova, Bridger, & Mecklinger, 2013). Sleep was expected to benefit associative but not item memory retention (Daurat, Terrier, Foret, & Tiberge, 2007; Drosopoulos, Wagner, & Born, 2005; Studte et al., 2015).

2. Methods

2.1 Participants

21 healthy young adults from Saarland University participated in this experiment. Data from 9 additional subjects were excluded due to (a) not sleeping (no occurrence of stage 2 sleep; n=3), (b) technical problems[1] (n=3) and (c) incorrect use of response buttons at pretest (n=3). The latter refers to two subjects who pressed two out of three possible buttons on at least 80% of all trials and one subject who consistently confused "old" and "recombined". All three of these excluded participants had a discrimination score at least 2 SDs lower than the mean in at least one of the two reward categories. The final sample consisted of 14 females and 7 males with a mean age of 21.7 ± 2.6. All participants stated that they did not have any sleep disorders, no known neurological problems and that they were right-handed (Oldfield, 1971). All gave written informed consent and were paid 20 € or equivalent course credit plus an additional reward which was dependent on their test performance (average: 9 € ± 3 €). The maximum additional reward was set to 20€.

2.2 Stimuli

270 semantically unrelated German word-pairs were used as stimuli. All words were nouns with a length between 3-10 letters and a frequency between 6 and 869 (Baayen, Piepenbrock, & Gulikers, 1995). 180 of the word-pairs were used in the previous nap sleep study from our lab (Studte et al., 2015). The remaining 90 word-pairs were newly created and evaluated in terms of semantic relationship and suitability to build a compound in order to reduce the pre-experimental associations within pairs (Bader et al., 2010). 30 additional

[1] This refers to two instances in which the sleep EEG recording did not work and a further instance in which E-prime failed to record responses so the session had to be stopped after the pretest.

subjects who did not participate in the main experiment rated the relatedness and unitization ability of the new and recombined word-pairs and only word-pairs with low relation and low unitization values (each ≤2 on a scale from 1-4) were included as test stimuli. There were six different stimuli-sets for word-pairs which were counterbalanced across our sample so that all items appeared equally often in each category (high/low reward; old/new/recombined). Recombined pairs were always rearranged within either the low or high reward category.

2.3 Design and Procedure

The experiment always began at 13:30 pm (see Fig. 1), at which time the sleep log - filled over the preceding three days - was checked by the experimenter. The sleep log asked for habitual bed, waking and rising times as well as for the occurrence of day naps and the ingestion of alcohol. Feelings of tiredness were also measured over several time points across the three days. Participants were instructed to maintain a normal sleep/wake pattern during the days before the experiment. At 13.45 pm the electrode setup began and the Handedness questionnaire as well as the Epworth and Stanford Sleepiness Scales were filled out. The *Epworth Sleepiness Scale* measures daily sleepiness by assessing the likelihood of falling asleep in different situations. The *Stanford Sleepiness Scale (SSS)* measures the current feeling of sleepiness on a 1-7 scale. There were 6 different time points for the sleepiness questionnaire, SSS1: before learning; SSS2: after learning SSS3: after pretest; SSS4: after napping; SSS5: before posttest and SSS6: at the end of the experiment.

- Insert Figure 1 around here -

The memory task was programmed using E-Prime 2.0 (Psychology Software Tools, E-Studio 2.0.8.90). Participants sat in front of the monitor at a viewing distance of about 65 cm.

Stimuli were presented in black on a grey background (maximal horizontal visual angle ≈ 5.7°). After a fixation cross (500 ms), reward symbols were shown for 1000 ms. Reward symbols were either € or €€€, the latter depicting the high- and the former the low-reward upcoming stimuli (see Fig. 2a). Participants did not know the exact value of either reward type (which was 0.20€ for high- and 0.02€ for low-reward correct answers) but were informed that the maximum additional reward they could earn was 20€, if they recognized all high-reward stimuli correctly at pre- and posttest[2]. Word-pairs were presented slightly below and above central vision at both study and test (vertical visual angle ≈4°). The presentation time of all word-pairs at study was 5000 ms. Participants were instructed to memorize items for a later memory test by imagining both items together in one picture. The study list with 180 word-pairs was divided into six blocks. There were self-paced breaks in-between blocks. Stimuli were presented in random order with an interval of 550 ms (of which 500 ms was a fixation cross). The duration of the study phase was approximately 26 minutes.

The initial memory test (pretest) was conducted immediately after the study phase. The pretest included 30 new, 30 old and 30 recombined word-pairs. Half of the test items had been associated with a high-reward cue during study, the remainder with a low-reward cue. Participants had to decide whether the presented word-pair was old, new or recombined and responded on one of three keys. The key assignment to right and left hand was counterbalanced across subjects. Word-pairs were presented for 1000 ms, followed by a 2000 ms long response window with an interval of 1000 ms. There were self-paced breaks in-between blocks. After the pretest, two electrodes were applied to the participant's chin to measure muscle activity during sleep, before they were asked to lie down at around 15:15 pm (±15 minutes). Participants were given the opportunity to sleep for a maximum of 90 minutes. After waking, participants watched 20-25 minutes of a movie (*Relaxing: The most beautiful landscapes on*

[2] It was made clear to the participants that low-reward stimuli contributed very little towards the additional 20€.

earth) featuring only instrumental sounds. This step was taken in order to reduce sleepiness effects on the second test (posttest). At around 17:15 (±15 minutes), the second test (posttest) was conducted. The posttest consisted of 60 new, 60 old and 60 recombined word-pairs; again half of these had been associated with high values during study, the other half with low values. The response procedure was the same as in the pretest.

- Insert Figure 2 a & b around here

2.4 Data acquisition and processing

2.4.1 Electroencephalogram (EEG)

EEG was recorded with BrainVision Recorder Version 1.20 (Brain Products). In total, 32 Ag/AgCl electrodes were used including electrodes which were located above and below the right eye and outside the outer canthi of both eyes in order to assess electro-ocular activity and 2 electrodes at the chin for electromyographic recordings. Data were recorded with amplifier band pass filter settings from DC to 100 Hz and a Notch-filter at 50 Hz. The sampling rate was 1000 Hz for polysomnographic data acquisition during the nap. The EEG data recorded during the pre- and posttest are not reported here. All electrodes were recorded referenced to the left mastoid electrode and re-referenced to the average of the left and right mastoid (offline). Electrode impedances were kept below 5 kΩ.

2.4.2 Sleep stage scoring

Preprocessing of the sleep data was conducted using BrainVision Analyzer (2.0, Brain Products). Each 30 second epoch of sleep was scored visually into rapid-eye-movement (REM)-sleep or non-REM (NREM) sleep stages 1, 2, 3 or 4 according to standard criteria (Rechtschaffen & Kales, 1968). Slow-wave-sleep was calculated as the sum of sleep stages 3 and 4. The time in minutes for each sleep stage, the total sleep time, the sleep onset latency,

wake time after sleep onset (WASO) and the percentage of sleep time in each stage with reference to total sleep time (TST) were determined.

2.4.3 Sleep spindle analysis

Sleep spindles were detected using an adaption of the algorithm originally provided by Ferrarelli et al., 2007 (see also Cox et al., 2012, Studte at al., 2015). In short, the envelope of the individual sleep EEG signal was computed using the Hilbert transform and its resulting absolute values. Unique thresholds for spindle detection were used for each participant. These were derived by calculating the mean plus two SD (lower threshold) and the mean plus four SD (higher threshold) of the participant's filtered EEG signal. The average envelope amplitude was examined for spindle-comprising sleep stages (2, 3, and 4). To classify a spindle, two criteria had to be fulfilled: i) the duration between the points at which the signal fell above and below the lower threshold needed to be at least 500 ms and ii) the signal also had to cross the upper threshold within this 500 ms time window (Ferrarelli et al., 2007). Spindle density (SpD) at electrode Fz was calculated for NREM (S2+SWS) sleep by dividing the number of spindles by minutes of NREM (S2+SWS) sleep.

2.5 Data Analysis

For the behavioral data, analyses of variance (ANOVA) with factors of reward (high/low), time (pretest/posttest) and item-type (item/associative) were used. An old/new discrimination Pr index (PrI- score) was calculated by subtracting false alarms to new pairs from the hit rate for old pairs ($PrI = hits_{old}\text{-}FA_{new}$) (Snodgrass & Corwin, 1988) and aimed to provide a measure of item memory. Of principal interest was the ability of participants to distinguish between old and recombined pairs, so an associative PrA-score was computed (Bader et al., 2010; Kriukova et al., 2013) to reflect associative memory. This was calculated

by subtracting the proportion of recombined pairs incorrectly classified as old (false alarms to recombined) from the hit rate for old pairs (PrA = $hits_{old}$ -FA_{rec}). For the reaction time data, ANOVAs with the factors time (pretest/posttest) and item condition (old_{high}/old_{low}/new/rec_{high}/rec_{low}) were conducted for correct answers. Subsidiary analyses were performed using t-tests which were corrected for multiple comparisons applying Holm's sequential Bonferroni correction (Holm, 1979). Only contrasts that survived correction are reported, except where noted.

For all analyses, the significance level was set to α=0.05. Where necessary, analyses included Greenhouse-Geisser corrections for nonsphericity with corrected p-values and uncorrected degrees of freedom (Greenhouse & Geisser, 1959).

3. Results

3.1 Behavioral data

- Insert Figure 3 around here -

Fig. 3 shows the mean PrI- (a) and PrA-scores (b) for the pre- and posttest separated by reward. To test the hypothesis that there will be a smaller decrease in memory performance from pre- to posttest for the high-rewarded compared to the low-rewarded word-pairs, a three-way ANOVA (with factors reward, item-type and time) was conducted. Main effects of time (F(1,20)=18.86, p<.001), item-type (F(1,20)=86.05, p<.001) and reward (F(1,20)=5.29, p<.05) and a marginally significant reward x time interaction (F(1,20)=4.26, p=.052) were revealed. To deconstruct the interaction, Bonferroni-corrected (p=.0125) follow-up tests were conducted, collapsed across item-type. At pretest, there was no significant reward effect (p=.447) whereas

this was significant at posttest ($t(20) = 3.413$, $p=.003$). The effect of time on performance was significant for low ($t(20) = 6.099$, $p<.001$) but not high-reward discrimination ($p=.188$).

For an overview, Table 1 shows the hit and FA rates as well as reaction times for pre- and posttest for each item- and reward condition.

For reaction times, an ANOVA with factors time (2) and item condition (5) on correctly responded to items, revealed only a main effect of item condition ($F(4,80)=49.19$, $p<.001$). Follow-up analyses revealed no difference in response times for high vs. low rewarded pairs within either the old or recombined categories (all $p>.23$). Participants responded faster to correct old responses than correct rejections and recombined pairs (all $p<.01$) as well as faster to correct rejections than recombined pairs (all $p<.01$) irrespective of reward category.

To explore whether there was an influence of sleepiness on memory performance at pre- and posttest, the subjective feeling of sleepiness (as measured with the Stanford Sleepiness Scale [SSS]) was subjected to an ANOVA for the 6 measured time points. A main effect of sleepiness over time was revealed ($F(5,100)=15.31$, $p<.001$). Participants felt most awake before (SSS1: 1.90±0.44) and after the experiment (SSS6: 1.38±0.5) as well as before the second test (SSS5: 2.14±0.85) and remained relaxed wakeful in-between (SSS2: 2.90±0.89; SSS3: 2.57±0.93; SSS4: 2.90±0.77). Participants felt more awake before the post (SSS5) than the pretest (SSS2) ($p=.012$, uncorrected). This latter effect argues against the possibility that sleepiness accounts for the decrement in memory performance from pre to post-sleep.

Table 1 Hit rates (%), FA rates (%) and reaction times (ms) for Pre- and Posttest

		Pretest			Posttest		
		Hit rate (SD)	**FA rate* (SD)**	**RT (SD)**	**Hit rate (SD)**	**FA rate* (SD)**	**RT (SD)**
Old	**High**	.71 (.19)	-	1486 (204)	.66 (.17)	-	1558 (181)
	Low	.65 (.19)	-	1509 (202)	.55 (.22)	-	1588 (171)
Rec.	**High**	.61 (.19)	.16 (.11)	1832 (268)	.58 (.19)	.15 (.12)	1873 (253)
	Low	.60 (.20)	.12 (.12)	1865 (273)	.51 (.16)	.17 (.14)	1852 (254)
New		.75 (.20)	.03 (.04)	1681 (202)	.65 (.17)	.05 (.07)	1720 (230)

*FA rate= old answers to new or recombined word-pairs

3.2 Sleep data

3.2.1 Polysomnographic data

A summary of sleep parameters is shown in Table 2. The average time spent in sleep was about 71 minutes, spent mostly in stage 2 (S2) sleep (43.56%). Participants showed on average about 15.60 minutes of slow-wave-sleep (22.52%) and about 3 minutes of rapid-eye-movement (REM) sleep (3.79%). Most participants showed SWS (n=18) but only one third reached REM sleep (REM: n=7) which accounts for the large variability of these measures.

Table 2 Sleep parameters

	Minutes	(SD)	%	(SD)
Latency	14.83	(12.22)		
Total Sleep Time	70.64	(15.83)		
Stage 1	8.14	(4.4)	11.56	5.93
Stage 2	31.52	(13.51)	43.56	12.68
Stage 3	10.36	(7.83)	15.04	11.61
Stage 4	5.24	(6.58)	7.48	9.45
REM	3.02	(4.92)	3.79	6.39
WASO	12.36	(11.19)	18.57	17.78

3.2.2 Sleep spindle data

Table 3 Sleep spindle correlations (Fz) with PrI/PrA scores at posttest

Low reward		**High reward**	
PrI	**PrA**	**PrI**	**PrA**
r=0.36 (p=.11)	r=0.3 (p=.19)	r=0.54 (p<.05)	r=0.52 (p<.05)
-	-	r=0.43 (p=.06)*	r=0.43 (p=.06)*

* Outcomes of partial correlation analyses with pretest performance as control variable

To test the prediction outlined in the introduction, correlations were calculated between SpD at Fz during NREM sleep (mean spindle density at Fz was 1.01, SD: 0.18) and Pr-scores for high-reward and low-reward pairs. As presented in Table 3 significant correlations were obtained between PrA_{high}-score at posttest and SpD_{NREM} as well as between PrI_{high}-score at posttest and SpD_{NREM} (Fig. 4). The corresponding correlations between SpD_{NREM} and PrA_{high}-score/PrI_{high}-score at pretest were not significant (p-values>.10), neither were there any significant correlations between SpD and PrA or PrI measures for low reward trials at pre- or posttest (p-values>.10). A partial correlation analysis revealed that the correlations between SpD and PrA_{high}-/PrI_{high}-scores at posttest were still marginally significant when pretest performance was controlled.

In previous studies of this kind, correlations between spindle density and overall memory performance at both pre and posttest have been reported (Gais et al., 2002; Studte et al., 2015), and this was also tested in the current data. SpD at Fz during NREM correlated significantly with overall memory performance (% correct responses for all word-pairs (old and recombined pairs in the low and high reward condition plus new pairs)) both before and after sleep (pre: r=0.44, p<.05; post: r=0.53, p<.05, Fig. 5). A partial correlation analysis (with pretest overall memory performance as covariate) revealed that the correlation between posttest overall memory performance and SpD during NREM is no longer significant (r=0.34, p=.14) when pretest performance is controlled for.

Taken together, the current data replicate previous findings that have shown that overall learning is related to NREM spindle density, but in addition reveal a specific correlation between NREM spindle density during napping and item and associative memory performance thereafter, which is unique to items tagged as motivationally salient during learning.

- Insert Figure 4 around here -

- Insert Figure 5 around here -

4. Discussion

The current study investigated whether different reward cues at encoding influence associative memory performance after nap sleep. Participants' memory for associations was tested after learning a list of word-pairs both before and after taking a nap. During learning, word-pairs were either preceded by a cue indicating a high reward for correct performance at test or by a low-reward cue. There is increasing evidence that sleep should preserve memories that are tagged as relevant for the future (Fischer & Born, 2009; Oudiette et al., 2013; Saletin et al., 2011; Stickgold & Walker, 2013; Wilhelm et al., 2011). Since high reward items should be of higher motivational value and therefore be tagged at encoding for selective consolidation during sleep (Stickgold & Walker, 2013)[3], we expected the memory benefit for high-rewarded pairs to be larger than for low-rewarded word-pairs after sleeping. This pattern was obtained: Memory performance declined to a greater extent for low rewarded than for high rewarded word-pairs after the nap. The absence of a wake control group in the current design, however, precludes any strong claims about the specific role of sleep on greater memory retention for high-reward items at posttest, on the basis of this pattern of data alone. We turn therefore, to the outcomes of the analyses on the relationship between sleep spindles and memory performance to provide important insight into the role of sleep in selective consolidation.

First, consistent with prior studies, we found a correlation between pre- and post-sleep overall memory performance and spindle density in NREM sleep. In one previous study, Gais and colleagues (2002) compared the influence of a learning experience (paired associate task)

[3] The neural mechanisms underlying tagging are still unknown. It has been reported that hippocampal activity at encoding is related to the amount of sleep related memory consolidation (Rauchs et al., 2011). However, it is still debated whether the hippocampus is the only brain structure involved in tagging or whether tags are generated in diverse neuroanatomical networks (Stickgold & Walker, 2013).

with a non-learning task - which was matched on all stimulus and task characteristics apart from the intention to learn - on sleep spindles in the following sleep episode. Sleep spindle density was found to be higher after the learning task compared to after the non-learning task, and spindle density was found to correlate with performance both before and after sleep. In the current study, overall memory performance both before and after napping was also related to spindle density. The findings of both studies may imply that consolidation during sleep is equally likely for all memories intentionally learned before sleep. Alternatively, the observation that memory performance before and after sleep correlates with spindle density could also suggest that individual differences in memory performance predict both sleep spindle density and post-sleep memory performance (Fogel & Smith, 2011). Regardless of which account is most appropriate, the link between sleep spindles and overall memory performance reported here supports the general claims of system consolidation theory concerning the role of spindles for memory retention (Rasch & Born, 2013).

Notably, however, a selective correlation between spindle density and high-reward memory scores at posttest was found in the current dataset as well. This relationship was not obtained for word-pairs in the low reward condition nor could the correlation between spindle density and high rewarded memories be accounted for by memory performance before sleep. This pattern supports the high relevance of sleep spindles for memory consolidation (Diekelmann & Born, 2010) and together with the behavioral data showing smaller decline for high than low reward from pre to posttest, these findings support the view that sleep enables the selective consolidation of memories from a specific learning experience. Other studies also report correlations between sleep spindles and specific memory measures post-sleep (Saletin et al., 2011; Schmidt et al., 2006). Saletin and colleagues (2011), for example, used a directed forgetting paradigm to investigate the role of explicit instructions during encoding on memory retention after sleep. It was shown that memory for to-be-remembered items was selectively

preserved after sleep, and that the memory performance difference between to-be-remembered and to-be-forgotten items was correlated with sleep spindle density. Our findings thus add to the converging evidence that learning instructions, intentions or other pre-sleep learning experiences can actively modulate memory consolidation.

Reward-related differences in memory performance were observable at post- but not pretest, which doesn't reflect patterns reported in some reports (Oudiette et al., 2013; Saletin et al., 2011). One reason for this outcome could be because the short interval between initial study and pretest was sufficiently short that working memory processes were available during pretest and may have obviated any reward effects on episodic memory. An alternative and not necessarily mutually exclusive possibility is that dopamine-mediated reward effects generally require a delay in order to be observed (Adcock, Thangavel, Whitfield-Gabrieli, Knutson, & Gabrieli, 2006; Feld, Besedovsky, Kaida, Münte, & Born, 2014; Wittmann et al., 2005). In line with these possibilities is the observation that where sleep studies have reported reward effects prior to sleep, the interval between learning and first test have generally been longer (i.e. 15-45 minutes) than in the current study (Oudiette et al., 2013; Saletin et al., 2011).

In contrast to our former study (Studte et al., 2015), we did not find sleep effects to be selectively related to associative memory retention. One possibility is that this is because the discrimination indices associated with item and associative memory (PrI and PrA) in the current study were derived from the same test phase. This step was taken in order to reduce overall memory load whilst maintaining sufficient trials to test reward effects. In our former study, however, two different memory tasks (single words vs. word-pairs) were employed in different test blocks to examine item and associative memory. Estimates of item and associative memory in the present study, therefore, are derived from the same response set which may have reduced the ability to detect dissociable effects of sleep on item and associative memory. Nonetheless, by finding larger effects of sleep on memory performance for high rewarded

word-pairs the data tally with prior reports of the beneficial effects of motivational cues on memory consolidation during sleep (Feld et al., 2014; Fischer & Born, 2009; Oudiette et al., 2013; van Dongen et al., 2012) and extend these effects to another form of reward-related learning.

In sum, the present study showed a differential influence of high- and low-reward associated cues on memory retention in that high-reward information was better retained after 90 minutes of nap sleep. Positive correlations between spindle density during NREM sleep and general memory performance pre- and post-sleep were found. Furthermore there were selective positive relationships between memory performance for highly rewarded word-pairs at posttest and spindle density during NREM sleep. These findings support the notion that processes during NREM sleep may be important for preferential consolidation of motivationally salient memories (Stickgold & Walker, 2013). This may indicate that reward cues induce tags (in a top down manner) for information that ensures these items are preferentially consolidated during sleep, leading subsequently to more durable memories.

Finally it is important to comment on the practical implications of these findings. Showing the importance of sleep for preserving associations between arbitrarily paired words that are tagged as relevant by moderate motivational cues, such as is often the case for items to be learnt for a vocabulary test, has important practical implications for educational settings, in particular for second language acquisition. The ability to learn arbitrary associations is critical across a wider variety of educational contexts (second language learning, face-name association to be learned in schools, kindergartens and other workplaces), however, and an intervention like nap sleep that promotes learning of previously unassociated information is thus of high relevance for the improvement and acceleration of learning for a range of contexts. The individual learner engaging in self-direct study may perhaps be best placed to apply the lessons learnt from the current data, given that they indicate that students do not need to work

late in the evening before sleep to benefit from the consolidation processes in sleep. A nap after learning or perhaps after a morning's revision for an afternoon test, may be as valuable as a night of sleep for consolidating newly learnt motivationally-relevant memories.

Acknowledgements

We gratefully acknowledge Roxanne Sopp, Stephanie Raschka and Lisa Riedel for assisting with data collection/analysis and Sebastian Hinrichsen for the development of the spindle detection algorithm. This research was supported by a grant from the German Research Foundation (IRTG 1457).

References

Adcock, R. A., Thangavel, A., Whitfield-Gabrieli, S., Knutson, B., & Gabrieli, J. D. (2006). Reward-motivated learning: mesolimbic activation precedes memory formation. *Neuron, 50*(3), 507-517. doi:10.1016/j.neuron.2006.03.036

Baayen, R. H., Piepenbrock, R., & Gulikers, L. (1995). The CELEX lexical database [Webcelex]. Philadelphia, PA, USA: University of Pennsylvania, Linguistic Data Consortium.

Bader, R., Mecklinger, A., Hoppstadter, M., & Meyer, P. (2010). Recognition memory for one-trial-unitized word pairs: Evidence from event-related potentials. *Neuroimage, 50*(2), 772-781. doi:10.1016/j.neuroimage.2009.12.100

Born, J., & Wilhelm, I. (2012). System consolidation of memory during sleep. *Psychological Research-Psychologische Forschung, 76*(2), 192-203. doi:10.1007/s00426-011-0335-6

Cox, R., Hofman, W. F., & Talamini, L. M. (2012). Involvement of spindles in memory consolidation is slow wave sleep-specific. *Learning & Memory, 19*(7), 264-267. doi:10.1101/lm.026252.112

Daurat, A., Terrier, P., Foret, J., & Tiberge, M. (2007). Slow wave sleep and recollection in recognition memory. *Consciousness and Cognition, 16*(2), 445-455. doi:10.1016/j.concog.2006.06.011

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

Drosopoulos, S., Wagner, U., & Born, J. (2005). Sleep enhances explicit recollection in recognition memory. *Learning & Memory, 12*(1), 44-51. doi:10.1101/kfg.mu-luebeck.de

Feld, G. B., Besedovsky, L., Kaida, K., Münte, T. F., & Born, J. (2014). Dopamine D2-like receptor activation wipes out preferential consolidation of high over low reward memories during human sleep. *J Cogn Neurosci, 26*(10), 2310-2320. doi:10.1162/jocn_a_00629

Ferrarelli, F., Huber, R., Peterson, M. J., Massimini, M., Murphy, M., Riedner, B. A., . . . Tononi, G. (2007). Reduced sleep spindle activity in schizophrenia patients. *Am J Psychiatry, 164*(3), 483-492. doi:10.1176/appi.ajp.164.3.483

Fischer, S., & Born, J. (2009). Anticipated Reward Enhances Offline Learning During Sleep. *Journal of Experimental Psychology-Learning Memory and Cognition, 35*(6), 1586-1593. doi:10.1037/a0017256

Fischer, S., Hallschmid, M., Elsner, A. L., & Born, J. (2002). Sleep forms memory for finger skills. *Proc Natl Acad Sci U S A, 99*(18), 11987-11991. doi:10.1073/pnas.182178199

Fogel, S. M., & Smith, C. T. (2011). The function of the sleep spindle: a physiological index of intelligence and a mechanism for sleep-dependent memory consolidation. *Neurosci Biobehav Rev, 35*(5), 1154-1165. doi:10.1016/j.neubiorev.2010.12.003

Gais, S., Molle, M., Helms, K., & Born, J. (2002). Learning-dependent increases in sleep spindle density. *Journal of Neuroscience, 22*(15), 6830-6834.

Greenhouse, S. W., & Geisser, S. (1959). ON METHODS IN THE ANALYSIS OF PROFILE DATA. *Psychometrika, 24*(2), 95-112. doi:10.1007/bf02289823

Holm, S. (1979). A Simple Sequential Rejective Multiple Test Procedure. *Scandinavian Journal of Statistics, 6*(2), 65-70.

Kriukova, O., Bridger, E., & Mecklinger, A. (2013). Semantic relations differentially impact associative recognition memory: Electrophysiological evidence. *Brain and Cognition, 83*(1), 93-103. doi:10.1016/j.bandc.2013.07.006

Lau, H., Tucker, M. A., & Fishbein, W. (2010). Daytime napping: Effects on human direct associative and relational memory. *Neurobiology of Learning and Memory, 93*(4), 554-560. doi:10.1016/j.nlm.2010.02.003

Marshall, L., Molle, M., Hallschmid, M., & Born, J. (2004). Transcranial direct current stimulation during sleep improves declarative memory. *Journal of Neuroscience, 24*(44), 9985-9992. doi:10.1523/jneurosci.2725-04.2004

Mednick, S. C., McDevitt, E. A., Walsh, J. K., Wamsley, E., Paulus, M., Kanady, J. C., & Drummond, S. P. A. (2013). The Critical Role of Sleep Spindles in Hippocampal-Dependent Memory: A

Pharmacology Study. *Journal of Neuroscience, 33*(10), 4494-4504. doi:10.1523/jneurosci.3127-12.2013

Murty, V. P., & Adcock, R. A. (2014). Enriched encoding: reward motivation organizes cortical networks for hippocampal detection of unexpected events. *Cereb Cortex, 24*(8), 2160-2168. doi:10.1093/cercor/bht063

Oldfield, R. C. (1971). THE ASSESSMENT AND ANALYSIS OF HANDEDNESS: THE EDINBURGH INVENTORY. *Neuropsychologia, 9*(1), 97-113. doi:10.1016/0028-3932(71)90067-4

Opitz, B., & Friederici, A. D. (2004). Brain correlates of language learning: the neuronal dissociation of rule-based versus similarity-based learning. *J Neurosci, 24*(39), 8436-8440. doi:10.1523/JNEUROSCI.2220-04.2004

Oudiette, D., Antony, J. W., Creery, J. D., & Paller, K. A. (2013). The Role of Memory Reactivation during Wakefulness and Sleep in Determining Which Memories Endure. *Journal of Neuroscience, 33*(15), 6672-U6758. doi:10.1523/jneurosci.5497-12.2013

Payne, J. D., Stickgold, R., Swanberg, K., & Kensinger, E. A. (2008). Sleep preferentially enhances memory for emotional components of scenes. *Psychol Sci, 19*(8), 781-788. doi:10.1111/j.1467-9280.2008.02157.x

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiol Rev, 93*(2), 681-766. doi:10.1152/physrev.00032.2012

Rauchs, G., Feyers, D., Landeau, B., Bastin, C., Luxen, A., Maquet, P., & Collette, F. (2011). Sleep contributes to the strengthening of some memories over others, depending on hippocampal activity at learning. *J Neurosci, 31*(7), 2563-2568. doi:10.1523/JNEUROSCI.3972-10.2011

Rechtschaffen, A., & Kales, A. (1968). A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Retrieved from

Saletin, J. M., Goldstein, A. N., & Walker, M. P. (2011). The role of sleep in directed forgetting and remembering of human memories. *Cereb Cortex, 21*(11), 2534-2541. doi:10.1093/cercor/bhr034

Schmidt, C., Peigneux, P., Muto, V., Schenkel, M., Knoblauch, V., Munch, M., . . . Cajochen, C. (2006). Encoding difficulty promotes postlearning changes in sleep spindle activity during napping. *Journal of Neuroscience, 26*(35), 8976-8982. doi:10.1523/jneurosci.2464-06.2006

Snodgrass, J. G., & Corwin, J. (1988). Pragmatics of measuring recognition memory: applications to dementia and amnesia. *J Exp Psychol Gen, 117*(1), 34-50.

Stickgold, R., & Walker, M. P. (2013). Sleep-dependent memory triage: evolving generalization through selective processing. *Nat Neurosci, 16*(2), 139-145. doi:10.1038/nn.3303

Studte, S., Bridger, E., & Mecklinger, A. (2015). Nap sleep preserves associative but not item memory performance. *Neurobiol Learn Mem, 120*, 84-93. doi:10.1016/j.nlm.2015.02.012

Tucker, M. A., Hirota, Y., Wamsley, E. J., Lau, H., Chaklader, A., & Fishbein, W. (2006). A daytime nap containing solely non-REM sleep enhances declarative but not procedural memory. *Neurobiology of Learning and Memory, 86*(2), 241-247. doi:10.1016/j.nlm.2006.03.005

van Dongen, E. V., Thielen, J. W., Takashima, A., Barth, M., & Fernández, G. (2012). Sleep supports selective retention of associative memories based on relevance for future utilization. *PLoS One, 7*(8), e43426. doi:10.1371/journal.pone.0043426

Walker, M., Stickgold, R., Alsop, D., Gaab, N., & Schlaug, G. (2005). Sleep-dependent motor memory plasticity in the human brain. *Journal of Cognitive Neuroscience*, 98-98.

Wilhelm, I., Diekelmann, S., Molzow, I., Ayoub, A., Molle, M., & Born, J. (2011). Sleep Selectively Enhances Memory Expected to Be of Future Relevance. *Journal of Neuroscience, 31*(5), 1563-1569. doi:10.1523/jneurosci.3575-10.2011

Wittmann, B. C., Schott, B. H., Guderian, S., Frey, J. U., Heinze, H. J., & Düzel, E. (2005). Reward-related FMRI activation of dopaminergic midbrain is associated with enhanced hippocampus-dependent long-term memory formation. *Neuron, 45*(3), 459-467. doi:10.1016/j.neuron.2005.01.010

Figure Legends

Fig. 1 Study design: Overview and timeline of the experimental procedure. The study phase consisted of the learning of 180 unrelated word-pairs. For the pretest 90 word-pairs (30 in each category, with 15 high and 15 low value pairs) were tested. The proportions of the different categories were the same at posttest, but for 180 unrelated word-pairs. The asterisks mark all measured time points of the Stanford Sleepiness Scale.

Fig. 2 Examples of a learning (a) and test trial (b) are presented. The violet arrow was not shown to the participants.

Fig. 3 Memory performance is shown for (a) PrI-scores (hits-FA_{new}) and (b) PrA-scores (hits-FA_{rec}) for pre- and posttest. Error bars show one standard deviation.

Fig. 4 Correlations are shown for PrA_{high}-/ PrI_{high} -score at posttest and spindle density (NREM) at Fz. These correlations remain marginally significant when pretest memory performance is treated as a covariate.

Fig. 5 Correlations are depicted for memory performance (in %) both before (a) and after sleep (b) and spindle density (NREM) at Fz. Memory performance (in %) includes the overall memory performance across all word pairs (old and recombined pairs in the low and high reward condition plus new pairs). The correlation between memory performance at posttest and spindle density (NREM) is no longer significant with pretest memory performance as covariate.

Fig 1.

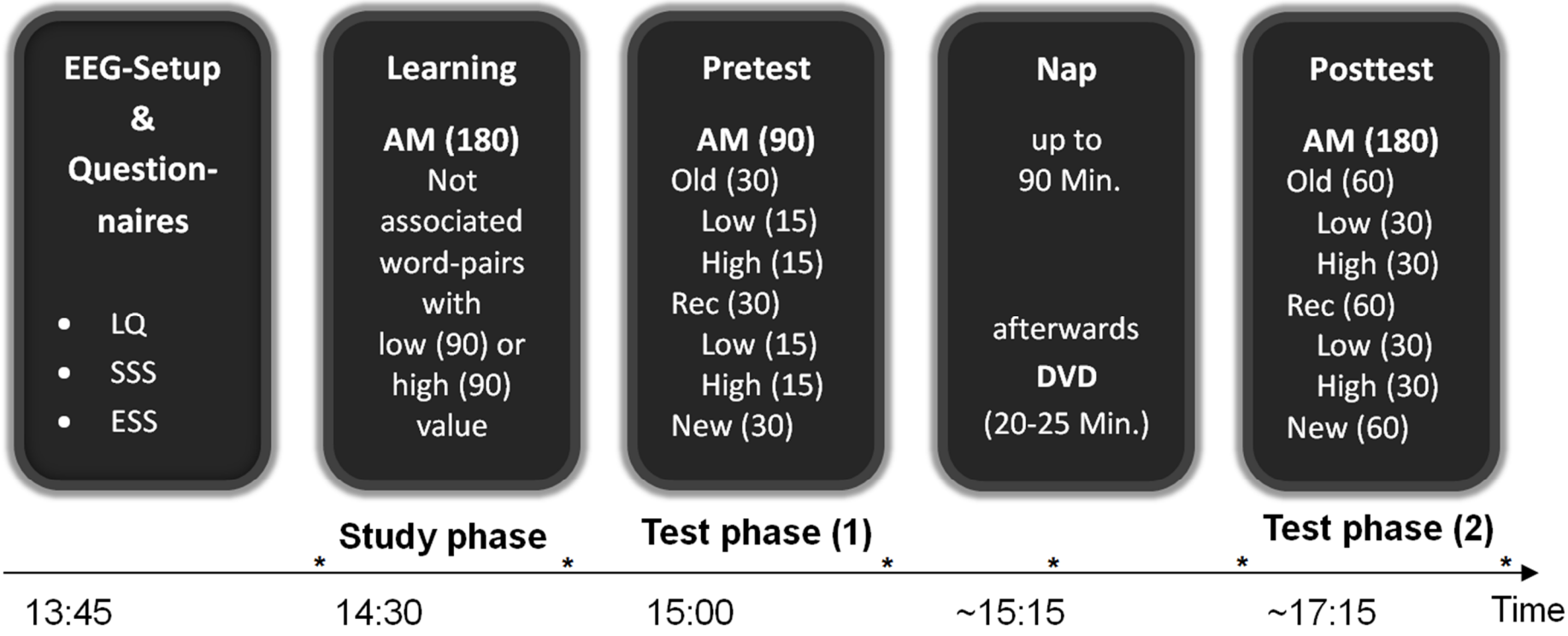

EEG-Setup & Question-naires
LQ
SSS
ESS
Learning
AM (180)
Not associated word-pairs with low (90) or high (90) value
Pretest
AM (90)
Old (30)
Low (15)
High (15)
Rec (30)
Low (15)
High (15)
New (30)
Nap
up to 90 Min.
afterwards
DVD
(20-25 Min.)
Posttest
AM (180)
Old (60)
Low (30)
High (30)
Rec (60)
Low (30)
High (30)
New (60)
Study phase
Test phase (1)
Test phase (2)
13:45
14:30
15:00
~15:15
~17:15
Time

Fig 2.

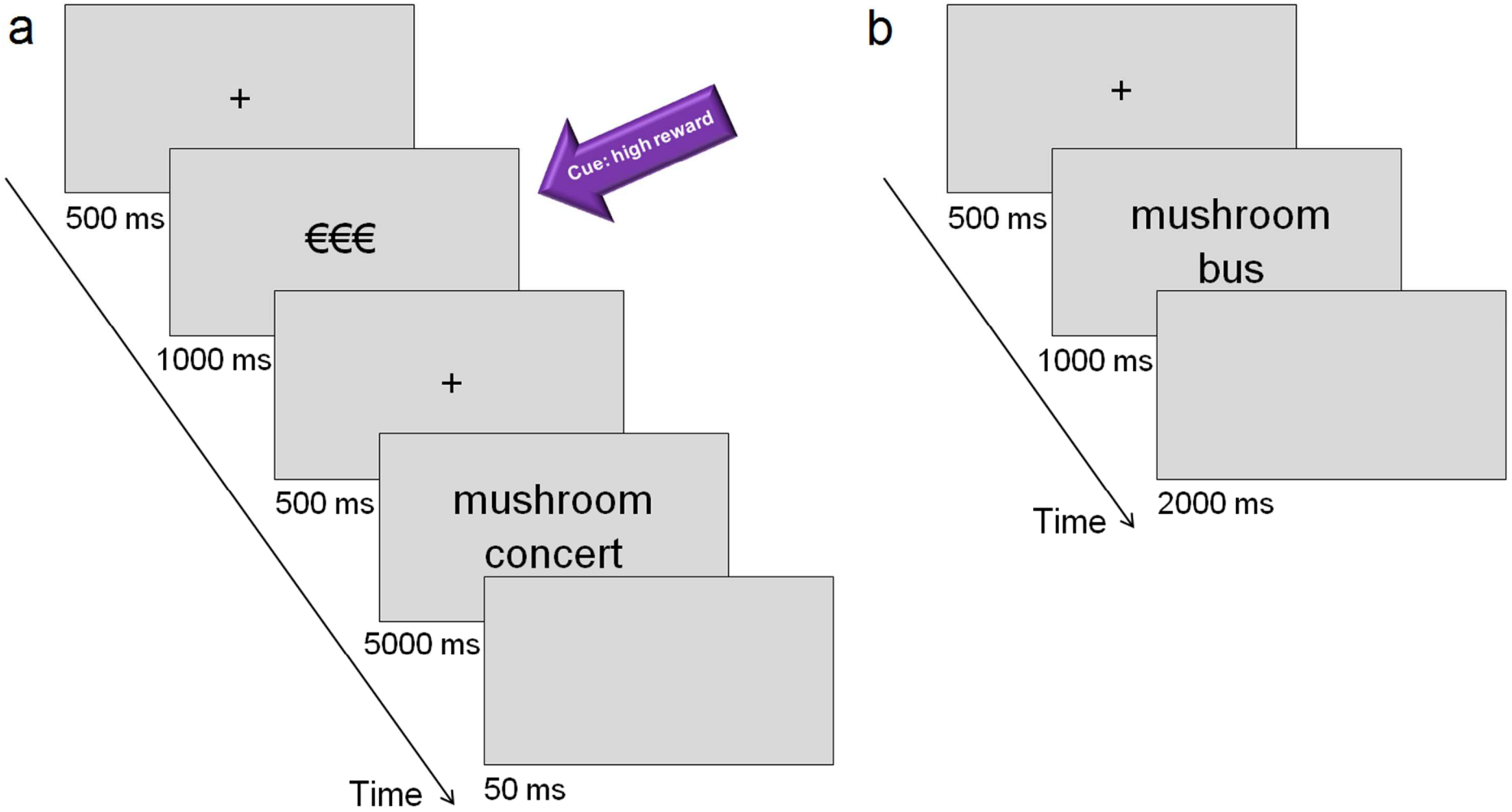

a
+
500 ms
€€€
Cue: high reward
1000 ms
+
500 ms
mushroom
concert
5000 ms
Time
50 ms
b
+
500 ms
mushroom
bus
1000 ms
Time
2000 ms

Fig 3.

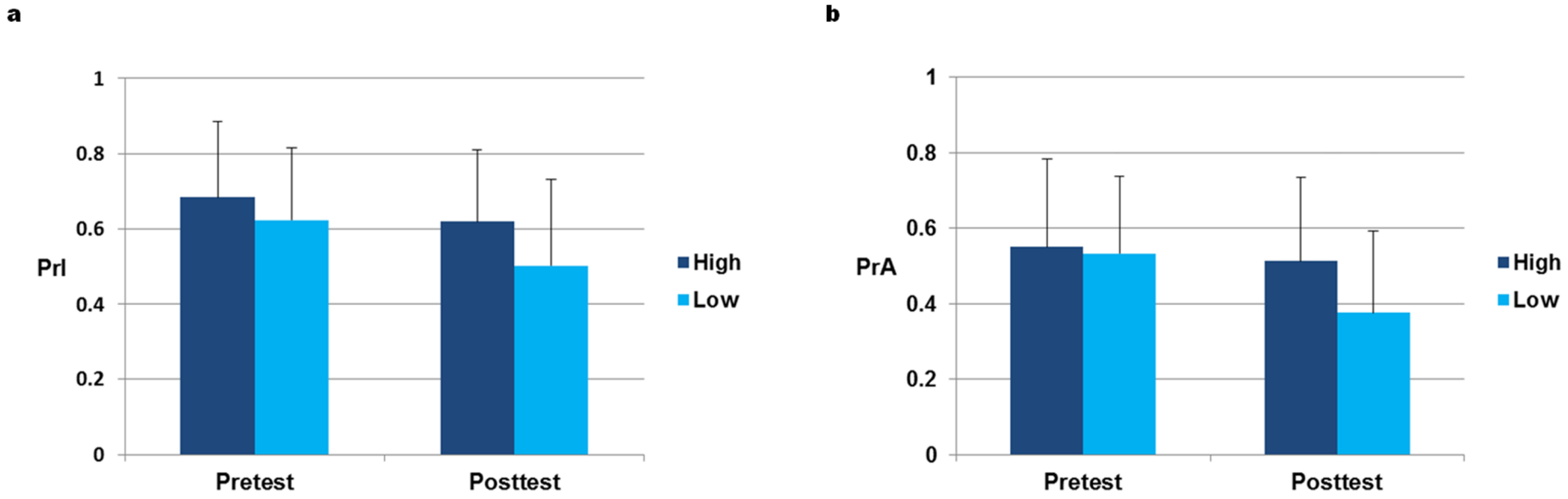

a
1
0.8
0.6
0.4
0.2
0
PrI
Pretest
Posttest
High
Low
b
1
0.8
0.6
0.4
0.2
0
PrA
Pretest
Posttest
High
Low

Fig 4.

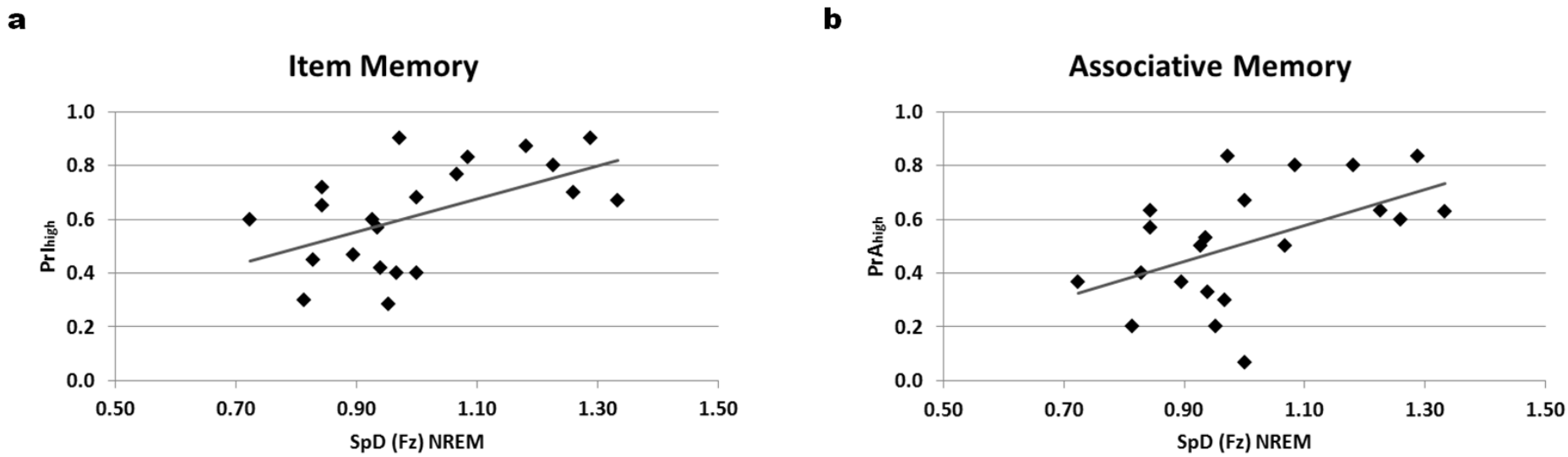
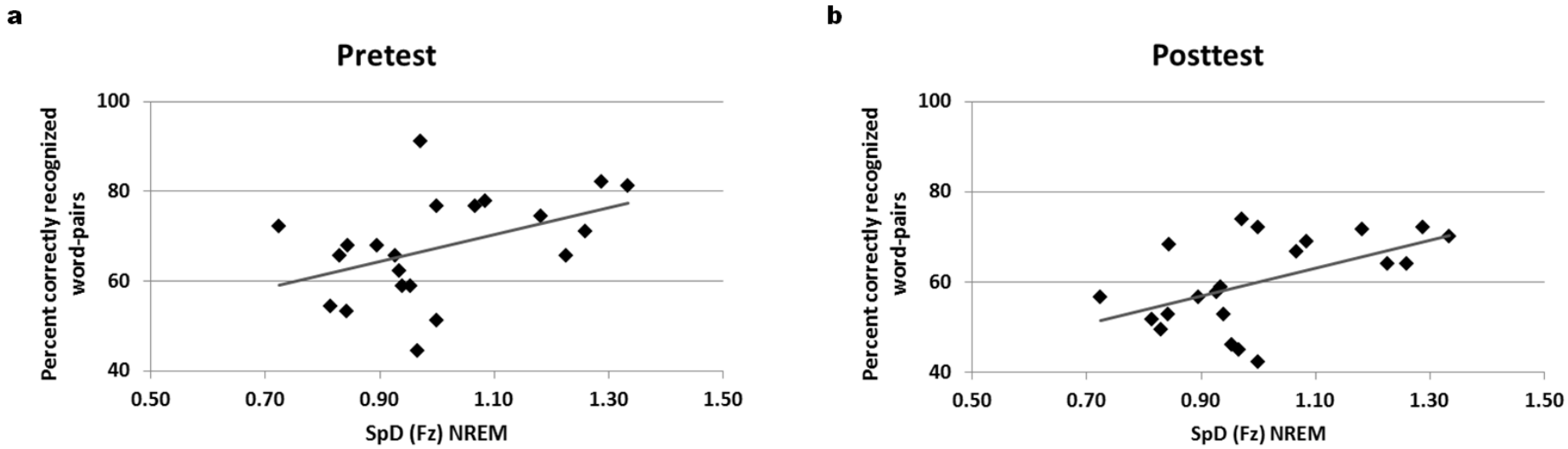

a
Item Memory
PrIhigh
1.0
0.8
0.6
0.4
0.2
0.0
0.50
0.70
0.90
1.10
1.30
1.50
SpD (Fz) NREM
b
Associative Memory
PrAhigh
1.0
0.8
0.6
0.4
0.2
0.0
0.50
0.70
0.90
1.10
1.30
1.50
SpD (Fz) NREM

Fig 5.


a
Pretest
Percent correctly recognized word-pairs
100
80
60
40
0.50
0.70
0.90
1.10
1.30
1.50
SpD (Fz) NREM
b
Posttest
Percent correctly recognized word-pairs
100
80
60
40
0.50
0.70
0.90
1.10
1.30
1.50
SpD (Fz) NREM